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PURPLE BLOTCH OF ONION (MACROSPORIUM PORRI ELL.)[1]

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INTRODUCTION

The disease of leaves, stems, and bulbs of onion caused by *Macrosporium porri* Ell. has received but little attention in the past. On the leaves and stems this organism seems to be, in the majority of cases, associated with the better known *M. parasiticum* Thüm.; and, perhaps on this account, the relative importance of these two fungi has been hitherto incorrectly estimated. During the progress of these studies the two organisms have been isolated from different sources and inoculation experiments performed under similar conditions. It now appears that *M. parasiticum* is but a secondary invader, as is also the case when it is associated with *Peronospora schleideni* Ung.

In Thaxter's (*31*)[3] description of the disease of onion leaves and bulbs caused by *Macrosporium porri* no common name was suggested. Nolla (*19*) in 1927 described it as a "purple spot of leaves." In the writer's judgment, however, the lesions on the leaves, stems, and bulbs are rather larger than is implied by this term. "Purple blotch" is, therefore, suggested as a name more nearly correctly descriptive of the disease and is recommended for general usage.

HISTORY AND DISTRIBUTION

The occurrence of a Macrosporium of the obclavate-spore type on the scales and blighted leaves of onion was first reported in 1890 by Thaxter (*31*), who collected specimens in Connecticut and Maine and identified the organism as *Macrosporium porri* Ell. Orton (*20*) reported the organism in Vermont in 1899, and Selby (*26*) noted that the leaf blight was especially noticeable in Ohio in 1898 and 1899. Chapman (*4*) in 1909 found on samples of onion seed in Massachusetts the spores of the so-called brown or black mold, which he attributed in part to this organism. Clinton (*5*) in 1904 reported it again in Connecticut, Stevenson (*28*) in Porto Rico in 1918, and Bruner (*3*) in Cuba in 1920. Blighted onion leaves collected in Java in 1919 by R. D. Rands and subsequently examined by the writer showed the characteristic symptoms of the disease, and the morphology of the spores coincided with those of American specimens. In 1921, Walker

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[3] Reference is made by number (italic) to "Literature cited," p. 486.

(*32*) reported a Macrosporium causing a rot of white sets in Wisconsin and Illinois. A culture of the causal organism supplied by him and studied by the writer was found to be identical with the one causing purple blotch. Chupp[4] in 1923 reported the disease from Massachusetts, New York, Louisiana, and Minnesota. Other reports indicate that it is generally distributed throughout the United States. During the summer of 1926 the writer found, in the vicinity of Racine, Wis., an organism causing numerous purplish lesions on the stems and leaves of Egyptian onion (*Allium cepa* var. *bulbellifera*) and Welsh onion (*A. fistulosum*). By comparison of its morphological and cultural characters with those of *M. porri* and successful cross inoculation on *A. cepa* its identity with *M. porri* was established. Nolla (*19*) in 1927 reported a disease of onions in Porto Rico caused by *Alternaria allii*. From his description there seems to be no doubt that the organism causing the disease was *M. porri*.

It should be noted here that some 10 years previous to Thaxter's publication this organism was first noted by Ellis on leaves of leek collected in New Jersey, and his description was published by Cooke and Ellis (*7*) in 1879. As will be shown later, the organism herein described as causing purple blotch on onion is also parasitic on leek (*Allium porrum*) and agrees with Ellis's description.

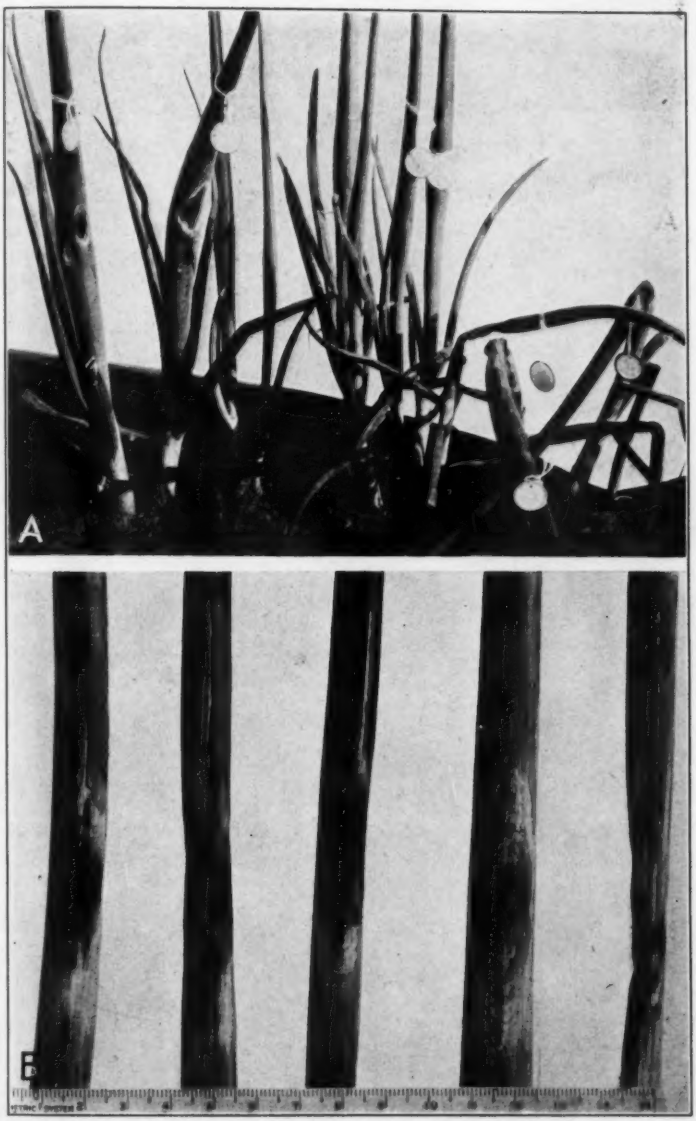
ECONOMIC IMPORTANCE

A survey of the literature on purple blotch of onions shows that the two distinct organisms (*Macrosporium porri* and *M. parasiticum*) are almost invariably associated, and in no case that has come to the writer's notice has any attempt been made to estimate separately the damage done by each or to evaluate their relative importance in the initiation of the disease. Selby (*26*) and others have at various times found them together on blighted onion leaves. Chupp[5] in 1923 reported that 50 to 75 per cent of the seed onions in Louisiana were attacked by them with a resulting loss of 30 to 50 per cent, but he did not indicate the extent to which each was responsible. Edgerton (*9*), however, in discussing the onion leaf blight in Louisiana made no mention of *M. porri*. Thaxter (*31*) was inclined to believe that it was the more truly parasitic organism. The writer's experiments in the greenhouse and in the field, the results of which are given in this paper, show that its germ tubes enter through the stomata of healthy leaves and seed stalks and produce lesions which rapidly cause their breaking down and death. Rapid decay also follows the introduction of the mycelium into wounds in the bulbs. On the other hand, experimental evidence obtained by the writer as well as by others shows that *M. parasiticum* is not even a facultative parasite.

It also seems desirable that outbreaks of the black-mold disease of onions should, in seed-growing districts especially, be more carefully investigated with a view to determing which of the two organisms, *Peronospora schleideni* or *Macrosporium porri*, is present. Care should also be exercised in distinguishing the conidia of the latter from those of saprophytic catenulate species of Macrosporium that may be present.

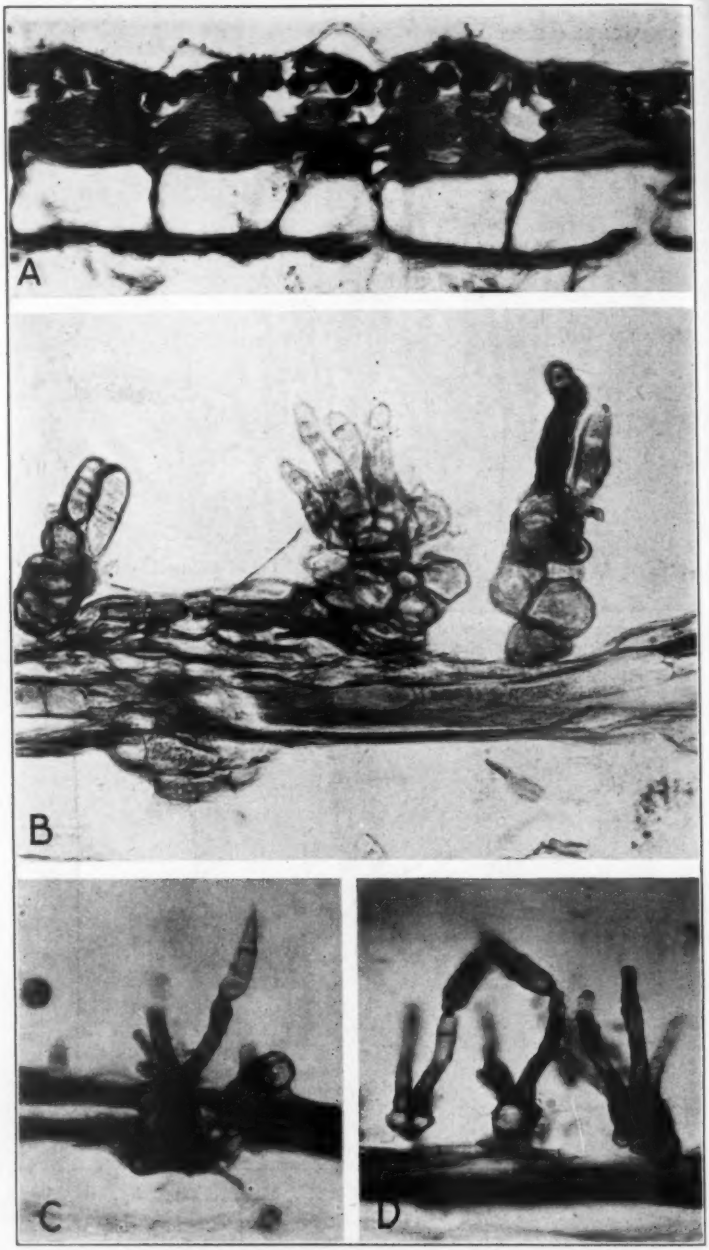
[4] CHUPP, C. DISEASES OF FIELD AND VEGETABLE CROPS IN THE UNITED STATES IN 1922. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Bul. Sup. 26, 163 p., illus. 1923. [Mimeographed.]

[5] CHUPP, C. Op. cit.



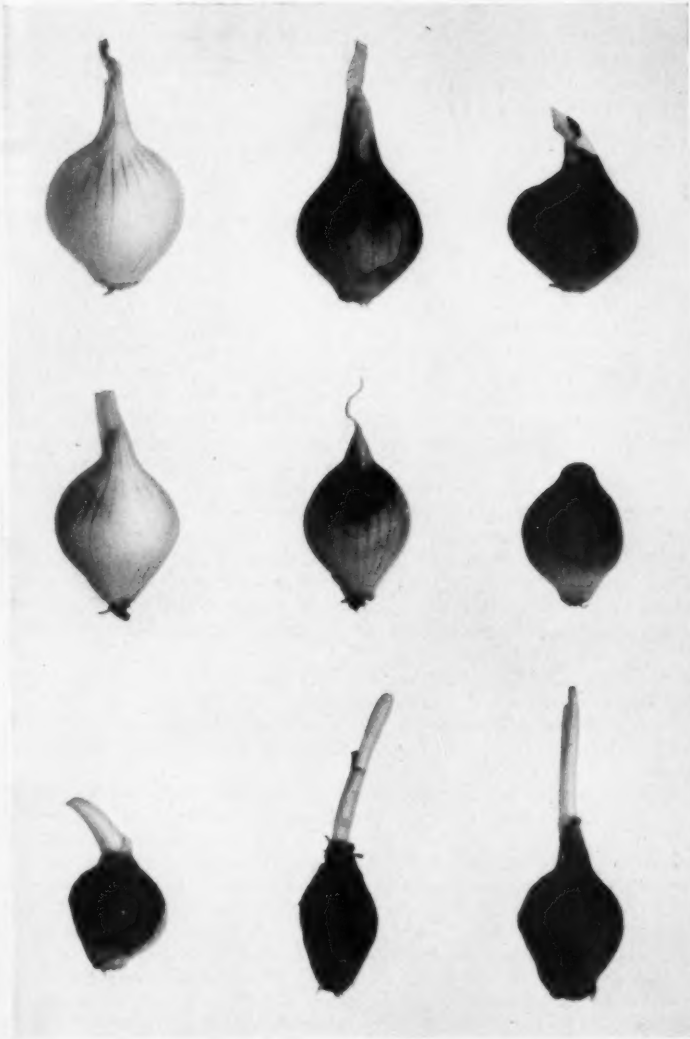
Purple Blotch on Onion Stems Caused by Macrosporium porri

A, Results of artificial inoculation. The two tagged stems at the left and the three at the right were inoculated through scalpel wounds; the two tagged stems in the center were wounded but not inoculated. Photographed 14 days after inoculation. Note the characteristic lesions on the plants at the left. Those at the right were so severely affected that the stems fell over. The controls remained free from infection. B, Lesions on onion stems resulting from natural infection. The fungus was fruiting abundantly in the central portion of each lesion. Note the tendency to zonation in the lesions. In some cases this was due to the concentric arrangement of the fascicles of conidiophores



Relation of Macrosporium porri to the Host Tissue

A, Photomicrograph showing hyphae in cross section in a subcuticular position. Note lamination of outer epidermal wall. (Cf. fig. 7 and text.) × 315. B, Photomicrograph showing sclerotioid masses of cells beneath the cuticle which has been ruptured by the development of the conidiophores. (Cf. fig. 1 and text.) × 390. C, Photomicrograph showing conidium attached to a conidiophore which has arisen from a sclerotioid mass of cells in a substomatal chamber. × 285. D, Photomicrograph of sclerotioid bodies immediately beneath the cuticle. Note conidiophores and attached conidia in section. × 285



Rot of white bottom sets (White Portugal variety) caused by *Macrosporium porri*. Naturally infected bulbs collected in the vicinity of Chicago, Ill., a few weeks after harvest. Two uninfected bulbs from the same lot are included for comparison. Infection usually occurs through the neck but occasionally through other wounds. The invaded tissue shows a semiwatery decay and becomes at first yellowish; the color gradually changes to reddish, then brownish, and eventually becomes almost black. Onion sets are often completely destroyed. In large bulbs only one or a few outer scales may be affected. Premature sprouting of infected bulbs is common.

From 1890, when Thaxter (*31*) reported the presence of *Macrosporium porri* on market onions, no mention of its occurrence on bulbs was made until 1921, when Walker (*33*) also reported it on the outer scales of white-onion sets. In another publication of the same year, Walker (*32*) very briefly described a Macrosporium (since found by the writer to be *M. porri*) as the cause of rotting of white-onion sets grown in Wisconsin and Illinois. By correspondence Ramsey reported that in the Chicago markets it is only occasionally found on onions. It must therefore be concluded that the loss incurred as a result of the rotting of bottom sets and of market onions by this organism is of minor importance.

SYMPTOMS

Lesions on the leaves and stems (pl. 1), whether occurring naturally or induced by artificial inoculations with spore suspensions, are, under favorable environmental conditions, easily visible to the naked eye within four days after inoculation. They are then grayish and deep sunken. After a few more days small dark spots appear in the centers of the lesions, later enlarging into purplish areas separated from the surrounding healthy tissue by narrow, light-colored zones, which may be more or less strongly marked. Two to four weeks later concentric light and dark zones appear over a part or the whole of the purple area, the darker ones being due to the dark-brown color of the numerous conidia present. At this stage the lesions may extend from 1½ to 4 inches longitudinally and partly or wholly around the stem or the leaf, which sooner or later falls over and dies. As will be shown later, the dark-purple color of the lesions is due at least in part to the radiating mycelium, which is subcuticular along the extending margins.

Infection of the bulbs (pl. 3) occurs in the autumn when weather conditions at harvest time are suitable. It usually takes place through the necks of topped bulbs or through other wounds made in harvesting. The disease develops rather quickly, especially during the earlier part of the season, producing a semiwatery decay. Secondary organisms readily invade the dead tissues and in some cases may obscure the true cause of the trouble. In a few weeks rotting of the scales is complete.

If neck infection has taken place, shrinkage of the infected succulent scale leaves results in loosening the dry outer ones. During the early stages the area affected by the disease may be limited, the shrinkage of the tissues causing unevenness of the two halves of the bulbs. This is easily evident on casual inspection and more so if median longitudinal or cross sections of the bulb are made. The color of the diseased area of red or brown onions may be drab of various shades, but that on white and yellow ones is distinctly reddish or yellowish. The color of sections of the diseased tissues may range from pale brownish to dusky drab. Sections made through or near the center of the diseased area reveal between the scales a dark, almost black, sclerotiumlike mycelial mass covering an area that is limited to about one-third of the periphery of the discolored region. Infected scales may be completely discolored and killed before the mycelium has invaded the tissues 1 cm. from the place where invasion began, and eventually they become dark brown or black. If infection is initiated

in a wound that affects only one scale, a dark lesion roughly circular in outline is formed. Surrounding this and involving the whole or a part of the scale is a yellowish area, the size of which depends on the length of time the disease has been in progress. On removal of the affected scale a mass of mycelium as described above appears underneath. All the succulent scales eventually decay. The thin ones in the center of the bulb, however, remain alive and usually begin to sprout, a symptom that is also associated with other onion rots, especially those due to *Botrytis allii* Munn and *B. byssoidea* Walker.

THE CAUSAL ORGANISM

MORPHOLOGY

Macrosporium porri was first noted by Ellis, whose description was published by Cooke and Ellis (*7*) and later emended and amplified by Thaxter (*31*).

MYCELIUM

The mycelium varies greatly on different substrata. On onion agar the diameters of the hyphae range from about 3μ to 14μ, in the

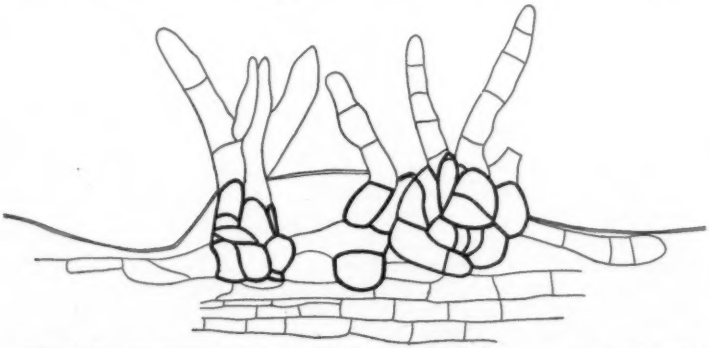


FIGURE 1.—Longitudinal section through part of a purple-blotch lesion on an onion stem showing beneath the cuticle the sclerotioid masses of cells with conidiophores arising from them. (Cf. pl. 2, B.) × 390

tissues of diseased bulbs, from about 2μ to 8μ, and in stems from 5μ to 10μ. The contents of the cells vary also with the substratum. In the host and on onion and potato-dextrose agars the cells are usually filled with highly refractive contents resemb ing oil droplets, whereas on media devoid of sugar, such as pea-meal and plain potato agars, they are vacuolate. On some artificial media, short, barrel-shaped cells occur occasionally. The color of the mycelium varies also with the medium. In the tissues of the host the mycelium is hyaline when young, but later it becomes dark brown; whereas on artificial media it may be of various tints and shades of yellow, pink, and gray.

CONIDIOPHORES

On the stems and leaves of the host the dark-brown unbranched conidiophores arise in fascicles from sclerotioid masses of cells. The lengths of 20 varied from 27μ to 45μ. Those produced on artificial

media are dark brown, not very greatly differentiated from the mycelium, and as a rule unbranched, although occasionally some occur with two, more rarely three, branches. They are 3 to 5 celled, the end cell sometimes being shorter than the rest.

Sclerotioid Bodies

After a period of two or three weeks of vegetative development during which the mycelium spreads rapidly, especially in the direction of the longitudinal axis of the stem or leaf, small sclerotioid masses of short, thick-walled cells of greater diameter than those of the normal mycelium begin to develop, generally subcuticularly, but sometimes in the substomatal spaces. (Fig. 1 and pl. 2, B, C, D.) They are of irregular shape, usually with a diameter of less than 50μ, rarely greater. From each arises a fascicle of usually about six conidiophores. They have not been observed in artificial cultures.

Conidia

Sporulation does not ordinarily occur in artificial media, but may be induced by the methods employed by Rands (*22*) and Kunkel (*16*). Even these may fail after some strains of the organism have been kept in culture for a few months. The Petri dishes containing the wounded cultures are laid open in sterile moist chambers (those used by the writer were 150 by 30 mm.), and within 48 hours abundant sporulation is usually secured under ordinary laboratory conditions. The conidia originate and develop in the same manner as those of *Macrosporium solani* already fully described and illustrated by Rands (*21*).

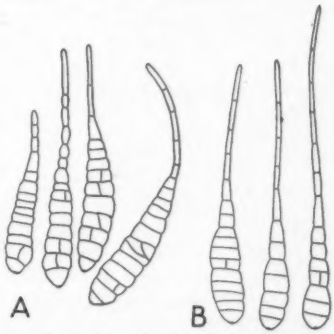


Figure 2.—Conidia of *Macrosporium porri:* A, From lesions on onion leaves; B, from artificial culture. Note the greater relative lengths of the beaks and the tendency toward fewer longitudinal septations. ×225

They are obclavate and are borne singly at the tips of the conidiophores. (Fig. 2.) On the host they have never been observed in chains, and in only two out of a few hundred cultures have a few been seen in chains of two. The main body of the conidium is brown and transversely and longitudinally septate. The number of transverse septations (including those in the beak) varies, the average in conidia borne on the host being 10 to 12. The beak is subhyaline and may be simple or forked, the latter condition occurring occasionally in cultures on artificial media. Nolla (*19*) reports beaked conidia from lesions caused by *Alternaria allii* (syn. *Macrosporium porri*). In conidia produced on onion and other agars the beak varies from one-third to two-thirds the total length of the conidium. Since, in germinating, its cells may produce germ tubes as do those of the main body of the conidium, the writer deems it an integral part, and in the making of measurements it should not be disregarded as has been done by some workers with other species of Macrosporium and Alternaria.

The conidia of *Macrosporium porri* from stem and leaf lesions vary from about 105μ to 220μ by 17.5μ to 26μ, and on artificial media (in this case onion agar) from 140μ to 370μ by 14.5μ to 33μ. On the basis of the measurements of 100 it may be stated that the majority of those from the host have a range of 130μ to 165μ by 21μ to 22.5μ, and those from artificial media 190μ to 290μ by 18.5μ to 22μ.

TAXONOMY

The Generic Names Alternaria and Macrosporium

On account of the apparent uncertainty in the application of the generic names Alternaria and Macrosporium it seems necessary to review briefly the original definitions and to refer also to a species of Macrosporium named by Berkeley (*1*). The genus Alternaria was first described by Nees (*18*) in 1817 with *A. tennis* as the type species. Muriform, attenuated, catenulate conidia were given as some of the characteristics of the genus. In 1832 Fries (*11*) described the genus Macrosporium and four species of it (two of which have been since transferred to Sporodesmium). The first of the four, the type species, *M. convalleriae*, has obclavate conidia borne in chains. Catenulation was, however, not given as a characteristic of the genus. Berkeley (*1*) in 1838 described a species, *M. sarcinula*, a type having sarcinaform conidia and therefore not falling within the limits of the genus Macrosporium as defined by Fries (*11*). His drawings show that a species of Macrosporium Fries was also present in his material. These conidia he thought were the immature form of *M. sarcinula*. In 1846 Fries (*12*) described the genus Alternaria, its limits corresponding to those of Alternaria Nees.

From this brief summary it is evident that fungi with the three following general types of conidia can be or have been with more or less justification referred to these two genera:

(*a*) Obclavate conidia typically borne singly; e. g., *Macrosporium solani* E. and M. (syn. *Alternaria solani* (E. and M.) J. and G.).

(*b*) Obclavate conidia borne typically in chains; e. g., *Alternaria brassicae* (Berk.) var. *macrospora* Syll. IV, No. 2613, 1886, Sacc. (syn. *M. brassicae* Berk. Smith Engl. F. 1836 Sacc. Mich. II, 1832).

(*c*) Sarcinaform conidia; e. g., *Macrosporium parasilicum* Thüm.

As most, perhaps all, of the fungi of the obclavate-conidium type bearing single conidia may under certain circumstances bear them in chains, it appears that the absence of catenulation is neither a constant characteristic nor one of sufficient importance to warrant generic distinction. Up to the present all efforts to find other morphological or physiological bases for distinction have failed. These two groups should therefore be included in the same genus.

Species producing sarcinaform conidia have also been placed in the genus Macrosporium by Berkeley (*1*), Thümen[6] and others. Examination of the illustrations and descriptions of these species by Miyabe (*17*), Bolle (*2*), and Tehon and Daniels (*29*) reveals that their conidiophores, the shape of the conidia, and the manner in which they are borne and developed are so distinctly different from the obclavate type that their inclusion in the same genus should not be continued unless Fries's definition is emended. Tehon and Daniels have, therefore, suggested a new generic name, Thyrospora, for fungi of

[6] Thümen, F. von. Mycotheca universalis [exsiccati]. No. 667. Klosterneuberg. 1877.

this type. Here it should be noted that echinulation of conidia, which they give as a characteristic of the genus, is not constant even in those produced in single-spore cultures. Smooth and echinulate conidia occur on the same thallus. With this disposition of the sarcinaform type of conidium, the names Alternaria and Macrosporium remain for the obclavate type whether borne singly or catenulate. According to the rules of the Brussels convention of 1910, Fries should be the authority for the genus that must consequently be Macrosporium. Elliott (*10*) disregarded this when he placed the fungi bearing obclavate conidia in the genus Alternaria Nees. Bolle (*2*) argued for the retention of Alternaria Nees on the ground that it was in more common use and for the continued use of Macrosporium Berk. for the sarcinaform type, although Berkeley did not describe this genus and did not seem to be aware of the wide differences between the two types. Reference to Saccardo (*25*) shows, however, that the name Macrosporium has been much more generally used than Alternaria, the species listed under the latter being very much in the minority. Only about 40 years ago did the name Alternaria come into prominence, and now the general trend in this country seems to be again toward Macrosporium. The fact that most of the economically important organisms are best known by the latter name may be adduced as additional justification for its continued use.

For fungi bearing obclavate conidia it is, therefore, proposed that the genus name Macrosporium Fries be continued in preference to Alternaria Nees.

Species of Macrosporium on Onion

The species of Macrosporium and Alternaria reported on *Allium cepa* are of two types, sarcinaform and obclavate spored. Representatives of the former are *M. parasiticum* Thüm. (sometimes referred to as *M. sarcinula parasiticum;* syn. *Thyrospora parasitica* (Thüm.) nov. comb.), *M. alliorum* Cooke and Mass. (*6*), and *M. cladosporioides* Desm. reported by Westendorp (*35*). These are so obviously different from the organism herein described that their further consideration is unnecessary. Of the obclavate type two species, *M. porri* and *Alternaria allii,* are recorded. The latter corresponds morphologically and physiologically with the writer's organism, which in turn agrees with the description of *M. porri* published by Cooke and Ellis (*7*) and by Thaxter (*31*) and with the latter's exsiccati. The reasons given by Nolla (*19*) for creating a new species, i. e., differences in size of conidia and the occasional occurrence of compound beaks, seem inadequate. His measurements agree closely with those given elsewhere in this paper, and the extremes of both fall on either side of those given in the original description. A great deal of variation in the size of conidia occurs in artificial media, and branched beaks are not infrequent under these conditions. This suggests that environment may be responsible for their occasional occurrence on the host. The evidence indicates that *A. allii* is synonymous with *M. porri.*

PHYSIOLOGY

Isolation of the Fungus

The pure cultures used during the first year of these investigations were obtained in 1925 from single-spore isolations from diseased leaves from Plainview, Minn. In the lesions there were two organisms,

Macrosporium porri and *M. parasiticum*. Four isolations of *M. porri* were carefully made according to Keitt's (*15*) method, and after comparison of conidial production in artificial culture one was selected. Later on other isolations were made from rotting onions obtained in the market at Madison, Wis. Small portions of the diseased scales were planted without previous sterilization, and the resulting growth proved indistinguishable from that of the strain isolated from the leaves. Sporulation was induced by Rand's (*22*) method, and single-spore cultures were made by Hanna's (*13*) dry-needle method. From these two series of isolations transfers were obtained, and with subcultures from them inoculations and other comparative studies were made. During the summer of 1926 other isolations, which were secured from lesions on the stems and leaves of Egyptian and Welsh onions, were found to be identical with the foregoing in cultural characters and parasitism.

Cultural Characters

Macrosporium porri grows well on all ordinary media, especially those in whose composition sugars are present. It does best on onion agar and on potato-dextrose agar, producing a vigorous growth of aerial mycelium. Pigmentation of the medium generally accompanies growth, the two being directly proportionate. One of the most interesting and variable characteristics of this organism is the great range of color it produces on different substrata. The pigment diffuses into the substratum, the intensity increasing with the age of the culture. Zonation and saltations sometimes appear. The former is more clearly marked on media containing less than 1 per cent of sugar. In testing cultures small sclerotiumlike masses of mycelium are sometimes formed along the sides of the tube. Some of them simulate perithecia, but all efforts to induce the organism to develop the perfect stage have been unsuccessful. The cultural characters have been studied upon a number of different media, but they will be described in detail only on onion agar.

On onion agar plates.—Conidia begin germinating at room temperature in one to two hours, producing on an average four to six germ tubes, which are hyaline at first, but later become dark. Thalli become macroscopic in 2 or 3 days and attain a diameter of 7 cm. in 8 days at an average temperature of 22° C. The color of the mycelium varies in different parts and becomes darker with age. Pigmentation of the medium begins after a few days and gains in intensity as growth proceeds until at 10 days it is brick red, changing later to a deep claret. Perhaps the most constant characteristic of Petri-dish cultures is the variability of the color of the aerial mycelium. Usually the central portion of the colony is dark-grayish olive, and around it is a zone of lighter colored mycelium about 1 cm. wide. In some cases the central portion of the thallus developed from a mycelial transfer is light gray; around this is a dark-grayish olive ring, and beyond it a lighter peripheral zone.

On other media.—Growth was studied on 15 different agars, on 10 different concentrations of dextrose with potato agar, on many modifications of Czapek's agar, on 4 liquid media, on 5 cooked substances, and on 8 kinds of fresh vegetables, a total of more than 60 substrata. On none of them was sporulation obtained without resorting to the special methods to which reference has already been made. The series of potato-dextrose agars and the modifications of Czapek's agar were employed in preliminary determinations of the principal factors involved in the production of color in the mycelium and pigment in the medium. In general, the results obtained on the different substrata indicate that on those devoid of sugar the growth is white or colorless, very little or no aerial mycelium is produced, and the medium is not discolored. In liquid media containing sugar no color is produced while the mycelium is developing beneath the surface, but in potato-dextrose decoction especially, color is rapidly produced very soon after aerial growth begins.

Comparative studies of *Macrosporium porri* and three strains of *M. solani* on cooked media (wheat, rice, starch, corn meal, and Lima bean) and on synthetic agars (Duggar's, Czapek's, and Cohn's) showed that in every case they were practically indistinguishable. On onion decoction rapid growth of *M. porri* was followed by diffusion of pigment into the liquid, which in three weeks became wine colored. On some of the fresh vegetables used growth was scanty and slow, but on slices of ripe tomatoes it was copious.

Production of Pigment

Throughout this investigation observations were made on the influence of various media, sugar, oxygen, temperature, light, rate of growth, age of culture, and reaction of the medium on the production of pigment. To summarize, it has been found that on sugar-free media no pigment is produced, but that all the sugars (hexoses) tried yield apparently equal amounts. Oxygen is essential, rate of growth and color production on potato-dextrose agar are directly proportional, and the optimal temperature lies between 24° and 28° C. Light appears to have no influence. The concentration of pigment increases with the age of the culture. Extremely acid and alkaline media are unfavorable for pigment production. From the variation noted on various substrata it is evident that the composition of the medium has a marked influence.

THE COLORING MATTER

A crude aqueous extract of the coloring matter becomes yellow on the addition of acid and pink on the addition of alkali. If a filtered aqueous extract of the color in potato-dextrose agar on which the organism has grown is allowed to stand for some weeks, it gradually loses its color with the formation of a dirty-yellow precipitate, part of which goes into solution on shaking with ether, chloroform, or xylol. The precipitate is also formed to a greater or less extent in pure cultures of the organism on potato-dextrose decoction. Practically all the coloring matter produced in cultures on potato-dextrose decoction is precipitated on the addition of neutral lead acetate. After filtration and washing of the precipitate with distilled water and subsequent treatment with 10 per cent hydrochloric acid, the coloring matter readily goes into solution in alcohol.

After evaporation of the alcohol the residue may be extracted with ether and then with alcohol. From the ether extract an orange-colored precipitate is obtained on the addition of petroleum ether. The precipitate after being washed dissolves in ether, chloroform, or alcohol, giving a solution whose color varies from yellow to orange red, depending on the concentration. On the addition of alkali the color changes to pink or red. With lead acetate its alcoholic solution gives an orange precipitate. In its reactions this ether-soluble pigment somewhat resembles some of the flavones of the higher plants. The alcohol extract in concentrated solution is dark brown. It is sensitive to changes in hydrogen-ion concentration.

Germination of Conidia

RELATION OF MEDIUM

The effect of various media on the germination of conidia was studied by using spore suspensions in hanging drops in Van Tieghem cells. Rain water, onion decoction, soil decoction, soil extract (steril-

ized and unsterilized), and distilled water were used. In the first four media named more than 90 per cent of the conidia germinated, but in distilled water the percentage was noticeably lower.

RELATION OF TEMPERATURE

To determine the relation of temperature to the germination of conidia and subsequent development of the germ tubes, drops of sterile water containing suspensions of conidia were placed on the surface of thin disks of water agar on cover glasses. These were inverted over Van Tieghem cells and the joints sealed with vaseline. The cells were put in moist chambers, and these were immediately placed in incubators running at the desired temperatures. It had been found in preliminary tests that germ tubes were very easily measured when the conidia were germinated in or on water agar, the conidia were easily located, and almost 100 per cent germination was the rule.

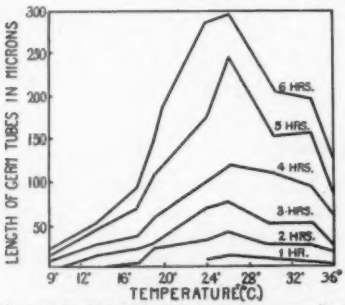


FIGURE 3.—Influence of temperature on production and subsequent development of germ tubes of *Macrosporium porri* on water agar

On an average, germination took place in about 45 minutes to 1 hour at temperatures between 28° and 36° C., in 1.5 hours at 24°, in 1.75 hours at 17.5°, in 2 hours at 13.5°, and in 3.5 hours at 9°. Conidia placed in Van Tieghem cells laid on a block of ice in the refrigerator showed no signs of germination in 6 hours, but germ tubes were subsequently produced.

At temperatures ranging from 13° to 28° C. the number of germ tubes produced by each conidium was generally higher than by those germinated at lower temperatures. In some strains they issued from any of the cells of the conidium except the last two of the beak. In one strain isolated from bulbs the beak itself was in some cases prolonged into a germ tube. As a rule the number of germ tubes varied from 2 to 6. At least 15 germ tubes produced at each temperature were measured at 1-hour intervals, and the average of these measurements is given in Figure 3. It will be seen that the optimal temperature for germination and germ-tube growth lies between 24° and 26°. At 36° germination took place, but after a few hours growth ceased. No further development took place on transference to room temperature. This observation was confirmed by the results obtained in the experiments on thallus development in which single conidia on onion agar were used.

In another series of experiments carried out under the same conditions except that meteoric water without agar was used, the percentage of germination was the same, but the time required was noticeably longer, especially between 28° and 36° C., throughout which range it was doubled.

EFFECT OF FREEZING AND DRYING

Sporulating cultures in Petri dishes and well-developed cultures on various media in test tubes were placed outside the window of the laboratory at Madison, Wis., in December, 1925, and brought in

again on March 21, 1926. During this period the thermometer registered as low as −14° F. Subzero (Fahrenheit) weather was experienced on two different occasions for periods of about three days each, and there were also others during which the temperature was above freezing for some days. On examination of the cultures it was found that over 50 per cent of the conidia had become detached from their conidiophores and that a great number of them had germinated and were attached to the dishes by germ tubes. With a dry needle single conidia still attached to their conidiophores were transferred to potato-dextrose agar plates. All germinated readily in the typical manner. Transferred mycelium, free of all spores, from the other cultures was also found to be viable. It can therefore be concluded that under Wisconsin conditions both the conidia and the mycelium of this fungus can survive the winter in pure culture. Other Petri-dish cultures were left in the incubator at 34° C. for over three months and then for a year under ordinary laboratory conditions, after which period the conidia readily germinated on agar.

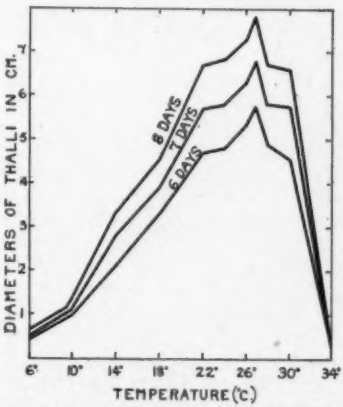


FIGURE 4.—Relative average growth of thalli of *Macrosporium porri* on onion agar at various temperatures

INFLUENCE OF VARIOUS FACTORS ON MYCELIAL GROWTH

LIGHT

Cultures of the organism in the laboratory have been exposed to varying light intensities, including direct sunlight shining through the window, diffused sunlight, electric light, and the total darkness of a photographic camera. Equally good growth and coloration of the substratum resulted under all these circumstances.

TEMPERATURE

For the study of the influence of temperature on growth of the mycelium, single conidia from onion-agar cultures or disks of mycelium were transferred to Petri dishes containing 25 c. c. of onion agar. The Petri dishes were left in moist chambers 150 mm. in diameter and 30 mm. high—each containing a few centimeters of water—in incubators at the desired temperatures. The diameters of the thalli were measured at six, seven, and eight days.

The results summarized in Figure 4 represent the average of four series of tests, in three of which the thalli were started with approximately equal-sized disks of mycelium. The total number of cultures measured was 112, the number at each temperature being approximately the same. The growth made by cultures held at the same temperatures was very nearly uniform. The optimal temperature lay between 22° and 30° C. The growth rate dropped

sharply between 30° and 34°. Between 34° and 36° the young hyphae died after a small amount of growth, visible only by transmitted light with the aid of a hand lens, had taken place. The original mycelium used in the transfer remained viable. The measurements of thalli grown at 6° and 10° were approximate, since the few hyphae present had to be examined with the aid of a hand lens.

ACIDS AND ALKALIES

The experiments outlined below and others of a similar nature were undertaken to gain a general idea of the degree of tolerance of acid and alkali by the organism, and no attempt was made to carry the work farther. The reaction of the medium during the growth period and at the end was, therefore, not determined.

The potato agar used was made with three-quarters of the usual quantity of water, the pH adjusted, and the dextrose (0.1, 1, and 2 per cent) dissolved in the quantity of water required to bring the whole to standard strength. Both the agar and the sugar solutions were then sterilized separately for 20 minutes at a pressure of 12 pounds. Then they were mixed, and approximately 25 c. c. of the mixture was poured into each Petri dish. Small portions of inoculum of equal size were placed in the centers of the dishes, and the resulting cultures were allowed to grow for seven days at 21° to 22° C. The results of several trials indicate that within the limits of pH 3.8 and pH 9 there is no appreciable difference in the rate of growth and in the character of the mycelium. The variation between different thalli on agar of the same pH was just as great as between those grown at each of the two extremes indicated. At seven days the average diameter of three cultures in Petri dishes poured with 0.1 per cent potato-dextrose agar was 6 cm. at pH 3.8 and pH 9, and that of six thalli at pH 7 was 5.9 cm. Measurements on onion agar made according to Munn's [7] formula and adjusted to different degrees of pH gave parallel results. The production and color of the pigment seem to be influenced to some extent by the degree of acidity or alkalinity of the medium. Beyond a certain point, the greater the amount of acid present the greater is the tendency to yellowish pigmentation, and the more alkaline the medium the less the amount produced. On extremely alkaline medium, color may be entirely lacking in the substratum.

SUGAR

In studying the growth of the organism on various media it was noticed that on plain potato agar the thallus was white and characterless, whereas on 2 per cent potato-dextrose agar it was beautifully colored and the medium deeply pigmented. As a result of this observation a limited series of experiments was undertaken to determine the percentage of sugar required for producing the greatest amount of pigment, and to find out whether the presence of sugar was the only factor on which its production depends. The results here outlined have also been found applicable to synthetic agars, such as Czapek's, and they indicate that the presence of sugar is an essential factor for the production of reddish pigmentation in onion agar, on blighted onion leaves, and on rotting white-onion bulbs attacked by

[7] Onion bulbs, 200 gm.; agar, 15 gm.; distilled water, 1,000 c. c.

this organism. Plain potato agar made according to the standard method, except that only three-quarters of the usual quantity of water was used in its composition, was divided into 150 c. c. portions in Erlenmeyer flasks after the reaction had been adjusted to pH 7.2, and then sterilized at a pressure of 10 pounds for 30 minutes. To each of them was added 50 c. c. of a similarly sterilized solution containing 4 gm. of either lactose, galactose, glucose, maltose, or saccharose, and Petri dishes were poured immediately, enough medium being used in each to insure that there would be no differences in growth owing to inequalities in the dishes. Growth and coloration on all these 2 per cent sugars were the same. To other flasks containing the same quantity of potato agar, 50 c. c. of sugar solutions of concentrations sufficient to make definite percentages of the resulting agar were added, and as in the preceding series Petri dishes were poured at once and approximately the same amount of mycelium transferred to each. When the thalli were 7 days old their diameters were measured and the amounts of aerial mycelium and of pigmentation compared. The optimal concentration of sugar for growth and coloration was found to be between 4 and 8 per cent. Greater or less concentrations were correspondingly less favorable. No pigment was produced on plain potato agar or on potato-dextrose agar containing more than about 16 per cent of sugar.

To determine whether the method of sterilization had any influence on pigment production, it was varied as much as was thought necessary. Both the potato agar and the sugar solutions were sterilized separately for one hour at 100° C. on three successive days and then mixed; in other cases they were mixed and then sterilized at a pressure of 15 pounds for varying lengths of time. As a result of a series of experiments, the conclusion must be drawn that the method of sterilization of potato-dextrose agar has no influence on the amount of pigmentation produced by the organism.

PATHOGENICITY

Inoculation Experiments in the Greenhouse

Details of the method used in isolating the organism have already been given. Either with or without the leaves being wounded, more than 50 healthy plants of *Allium* spp. grown in the greenhouse from sets were at various times inoculated with mycelium or conidia from pure cultures. The method of inoculation with conidia is described elsewhere. In cases where mycelium was used as inoculum it was either introduced into small scalpel wounds or placed between or on the uninjured leaves or stems of the plants. When wound inoculations were made the plants were either left under ordinary greenhouse conditions or placed in moist chambers for three or four days. On the other hand, if inoculations were made without wounding the tissues the plants were invariably placed in moist chambers for the same length of time. In both cases the symptoms already described began then to appear practically without exception. When the lesions had further developed, the causal organism was easily and repeatedly reisolated after sterilization of the surface of the diseased tissue for 30 seconds with a 1:1,000 solution of mercuric chloride, followed by washing with sterile water. All the check plants, numbering more than 20, remained healthy.

The two tested species of Allium, including white, yellow, red, and brown varieties of onion (*A. cepa*) and leek (*A. porrum*), were equally susceptible.

Inoculation Experiments in the Field

During the summer of 1926 several series of inoculation experiments were performed in a small plot of red, brown, yellow, and white varieties of onion grown in the vicinity of Racine, Wis. In each series from 5 to 10 plants were inoculated and 2 or 3 used as checks. It has already been noted that after a few months in pure culture *Macrosporium porri* failed to sporulate even when wounded; therefore the first inoculations were made with mycelium, which was inserted into scalpel wounds. Sporulation on the host began within a few weeks after inoculation, and single-spore isolations therefrom sporulated after being wounded and treated according to the methods already outlined. Subsequent inoculations were made with suspensions of conidia obtained from the lesions on the host, and, as will be seen in the more detailed reports that follow, they were uniformly successful. In all cases checks remained healthy.

The first inoculations were made on June 9 by wounding the leaves and stalks of seed onions with a scalpel and inserting some mycelium from pure cultures. A wick of absorbent cotton dipped into a dish containing distilled water and wound loosely around the plants was used to keep the surface moist during the first three days following inoculation. Symptoms were noted on June 15, and on June 24 the largest lesion was 1½ inches long. Sporulation began on July 6, on which date one lesion extended 4 inches longitudinally.

On June 18 another series of wound inoculations was made. On June 24 symptoms appeared, and on July 6 two of the stems were partly and three others completely girdled. Sporulation was noted on some lesions on July 8 and on others on July 14.

On July 6 conidia produced on the lesions from the inoculations of June 9 were washed off with distilled water and sprayed with an atomizer on healthy unwounded flower stalks. Development of the few lesions resulting was at first slow but later rapid, resulting in the breaking down of the infected stalks by September 1. On July 30, inoculations with conidial suspensions were again made, and, as on the previous occasion, humid conditions were maintained by means of a wick of absorbent cotton dipped in distilled water and wound around the plants. The weather was particularly favorable for infection, the thermometer registering between 60° and 65° F. (15° to 18° C.), while light showers on this and the following days helped to keep the air almost saturated with water vapor. On August 5 the resulting lesions were plentiful and well advanced. Further development was rapid, and at the end of four weeks the inoculated stalks were all dead. Other inoculations were made on August 12 with suspensions of conidia obtained from lesions on Egyptian and Welsh onions, respectively. The resulting lesions were in all respects similar to those previously produced.

Inoculation of Bulbs

Wound inoculations of more than 100 bulbs of white, yellow, red, and brown varieties of onions also were successful in all cases. The bulbs were immersed for five or more minutes in a 1:1,000 solution

of mercuric chloride and washed in sterile water, and scalpel wounds were made either in the necks or in the outer scales. They were then inoculated with mycelium from pure cultures, sealed in some instances with paraffin, and put in oiled-paper bags. After two weeks the organism was reisolated repeatedly from the rotting bulbs either with or without disinfection with mercuric chloride solution and its presence in the tissues demonstrated by free-hand and microtome sections. More than 50 checks were used, and all remained healthy.

To make doubly sure that inoculation was alone responsible for the rotting of the bulbs, parts of single scales were sterilized with 1:1,000 mercuric chloride solution for 30 seconds, washed in sterile water, placed in sterile Petri dishes, and inoculated with mycelium from pure cultures. The typical aerial mycelium of the fungus, the reddish discoloration of the scales, the presence of the hyphae in the tissues, and the reisolation of the organism with and without previous external disinfection leave no doubt whatever that *Macrosporium porri* is a parasite capable of infecting onion bulbs through wounds. After establishing itself in the wounded scales, invasion of the inner uninjured scales follows.

Relation of Temperature to Bulb Rot

On reference to the relation of temperature to mycelial growth it will be seen that the organism grows extremely slowly at 6° C., that between 10° and 22° it increases rapidly, that 27° is optimum for it, and that 34° is the maximal temperature at which it will grow. Small bulbs of different varieties of onion were sterilized, wounded in the necks, inoculated with mycelium, placed in oiled-paper bags, and left at temperatures ranging between 4° and 34°. It was found that at various temperatures the degree of rotting was, as nearly as could be estimated, proportional to the rate of growth of pure cultures of the organism held at corresponding temperatures. Little or no decay was perceptible in bulbs held at 4°. The decay at 14°, though moderate, was comparatively and in many cases altogether free from contaminating organisms, which at higher temperatures almost invariably appeared in spite of the exercise of great care in disinfecting the bulbs before inoculation. Under ordinary laboratory conditions rotting was complete in two weeks. Above 22° decay was more rapid, but the presence of other organisms made it difficult to estimate to what extent *Macrosporium porri* was responsible.

RELATION OF THE PARASITE TO THE HOST TISSUE

METHODS

For the study of penetration, onion plants a few weeks old, grown from sets in pots in the greenhouse, were placed in a moist chamber, distilled water was sprayed on the leaves with an atomizer, and conidia were carefully applied with a needle to selected drops on the leaves. Essentially the same procedure was followed with stems of seed onions grown out of doors. Neither leaves nor stems were injured either previous to, during, or after the application of the conidia. Forty-eight hours afterwards portions of the tissues were killed and decolorized in 95 per cent alcohol, placed on slides, and a few drops

each of lactophenol and of 2 per cent aqueous solution of cotton blue stain applied. A cover glass was put on and the preparation examined in toto under the microscope. At the same time other portions were killed and fixed either in 95 per cent alcohol or in formol-acetic alcohol, dehydrated, embedded in paraffin, and sectioned 10μ thick. Cross and longitudinal sections were stained in a 2 per cent solution of safranine in 50 per cent alcohol for 12 hours and counterstained with a 2 per cent solution of light green in absolute alcohol.

Material obtained during the summer of 1926 from naturally occurring lesions on the stems of Welsh and Egyptian onions and from others artificially produced on leaves and stems of some of the varieties of common onion was used for the study of the relation of the parasite to the leaf and stem tissue. Portions of the lesions were fixed, dehydrated, infiltrated with paraffin, sectioned, and stained as outlined in the preceding paragraph.

Since naturally infected bulbs were rarely available during the period in which these studies were in progress, the material used was taken

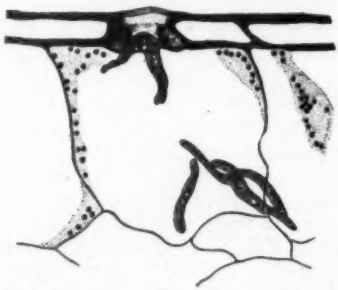


FIGURE 5.—Section of onion leaf showing portions of germ tubes of *Macrosporium porri* in a substomatal chamber entering an adjoining cell by direct penetration of the wall. × 190

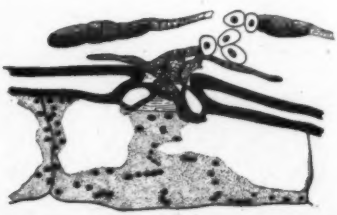


FIGURE 6.—Germ tubes of *Macrosporium porri* issuing from a spore (shown in cross section) and entering through a stoma of an onion leaf. × 190

from some that had been artificially inoculated through wounds. Portions of affected scales and of those immediately beneath were fixed in Gilson's fluid or in formol-acetic alcohol, washed, dehydrated, embedded in paraffin, and sectioned 8μ to 10μ thick. The differential stain recommended by Dickson (*8*) gave such good results that it was employed almost exclusively.

PENETRATION AND SUBSEQUENT DEVELOPMENT

Under ordinary conditions in the greenhouse and in the field, germination of spores on the leaves was observed to begin within three hours, and after the germ tubes grew for a few hundred microns they entered through the stomata. (Figs. 5 and 6.) Stomatal penetration has been observed repeatedly in in toto preparations and in paraffin sections. The hyphae may develop to a greater or less extent in the substomatal chambers, sometimes filling them, and sooner or later penetrate the underlying tissues either intercellularly or intracellularly. At the same time other hyphae grow through the lumina of the epidermal cells into the thick outer wall. Here they run parallel with the cuticle, at first separated from it by a thin layer of tissue

which soon disappears, leaving them in a subcuticular position. Several layers of mycelium may be produced beneath the cuticle, the epidermal cell walls in the meanwhile become laminated and the middle lamellae weakened. The hyphae then grow between the laminae, forcing them farther apart, then between and through the cells into the underlying tissues. After a period of two to three weeks

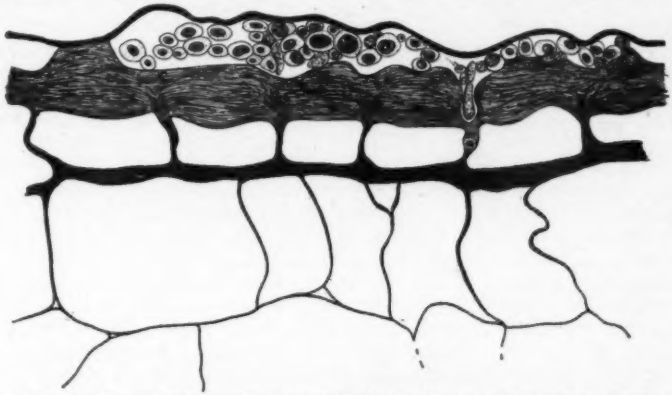


FIGURE 7.—Cross section of an onion stem (seed stalk) showing the development of hyphae of *Macrosporium porri* in a subcuticular position and also the lamination of the outer epidermal wall. (Cf. pl. 2, A.) × 315

of rapid vegetative development, small sclerotiumlike masses of thick-walled cells are formed subcuticularly, or in the substomatal chambers. (Figs. 1 and 7, pl. 2, A.) From them the conidiophores arise in fascicles, rupturing the cuticle, which ordinarily remains intact up to this stage of the development of the disease.

It has already been pointed out that the mycelium forms sclerotiumlike masses between the scales of the bulbs. How it penetrates the underlying scales has not been determined, but, once through, the mycelium ramifies between it and the outer walls of the epidermal cells. (Fig. 8.) The discoloration of the tissues well in advance of the invading hyphae indicates the production of enzymes, which soften the middle lamellae and the cell walls. After the walls of the epidermal cells have been penetrated, the mycelium spreads through the tissues intercellularly and intracellularly. The cell contents soon disappear. Lamination of the walls occurs. The mycelium has been seen not only in the epidermis and parenchyma but also in the phloem and the xylem. After a time the parenchyma becomes filled with the mycelium, the walls later disappear, and the whole scale becomes in effect a mass of dark-brown or black mycelium.

FIGURE 8.—Cross section through an infected bulb scale showing intracellular and intercellular hyphae. × 90

COMPARISON WITH MACROSPORIUM PARASITICUM

Reference has already been made to the relative importance of *Macrosporium parasiticum* and *M. porri* where the two are found in the same lesions. All the evidence establishes the fact that *M. porri* is a parasite, whereas, on the other hand, all workers but one on this problem agree that *M. parasiticum* is merely saprophytic. Miyabe (*17*) inoculated all parts of onion leaves with the conidia of the latter, but in no case was infection of the green leaves obtained. Only two infections on the sheaths of the leaves resulted. Thaxter (*31*) failed to produce the disease by inoculation with conidia. Shipley (*27*) and Bolle (*2*) found the organism only in plants that had been attacked by *Peronospora schleideni*. Bolle (*2*) made inoculations in the greenhouse and field and got negative results. The writer during the late summer of 1926 found the organism in abundance on plants attacked by *P. schleideni* in a field near Milwaukee, Wis., and also on lesions caused by *M. porri* on Egyptian and Welsh onions. Teodoro (*30*), however, claimed that he secured stomatal penetration on onions grown at Madison, Wis. Two comparative series of wound inoculations with *M. porri* and *M. parasiticum* were made by the writer on the leaves and stems of *Allium cepa*. One series was made in the greenhouse and the other outdoors. In each series six plants were inoculated with each organism. Mycelium from single-spore cultures of *M. parasiticum* from diseased specimens obtained from Madison and Milwaukee, Wis., and from Mississippi was used as inoculum. In no case did infection result, whereas inoculations with *M. porri* at the same time and under the same circumstances were all successful. All four checks in each series remained healthy. The association of these two organisms is comparable to that obtaining in the early blight of potato where *M. solani* is the organism causing the disease and *Alternaria fasciculata* the accompanying saprophyte.

COMPARISON WITH MACROSPORIUM SOLANI

Early in the progress of these investigations the close similarity of *Macrosporium solani* and *M. porri* suggested their possible identity. Parallel series of cultures of the former organism obtained from Maine and Wisconsin and strains of the latter isolated by the writer were indistinguishable in appearance. The variation between strains of the same organism was as great as that between the two species. Neither species produced conidia in culture except when the mycelium was wounded. The conidia were indistinguishable in size and character. The measurements of conidia of *M. solani* by Jones and Grout (*14*), Rands (*23*), and Rosenbaum (*24*) are almost identical with those of *M. porri* (syn. *Alternaria allii*) obtained by Nolla (*19*) and the writer. In both organisms branched beaks and occasional chains of two conidia occurred in artificial culture. The relation of temperature to germination of conidia of *M. solani* as given by Rands (*21*) and that of *M. porri* as given in these pages shows a marked similarity. Only in the parasitism of the two organisms was there a marked difference. Parallel inoculation experiments were made on four unwounded potato plants in the greenhouse, with the result that those made with *M. solani* were in every case successful, while

those with *M. porri* were not. The latter organism, however, produced very small lesions when wound inoculations were made, but no trace of mycelium could be found in the tissues. Inoculation of 12 onion bulbs with *M. solani* was also tried, but without success, while those inoculated with *M. porri* and placed under the same conditions were rotted in two weeks. Both organisms produced large lesions on ripe tomatoes when wound inoculations were made.

Physiologic specialization is already well known in the fungi. Perhaps the example most frequently studied is *Puccinia graminis* Pers., which on the basis of its ability to attack different species of the grasses and varieties of the same species, has been divided into subspecies and races. *Macrosporium porri* and *M. solani* are almost indistinguishable in their morphological and physiological characters; but they differ in their parasitism, the former attacking species of the Liliaceae and the latter species of the Solanaceae. Both can cause lesions on the ripe fruit of the tomato. Are they to be regarded as different species, or as races of the same species? A critical study of the two organisms with a view to inquiring into the bases of their physiologic specialization is desirable.

CONTROL

Purple blotch of bottom sets is usually of minor importance in the onion-growing districts of Wisconsin and Illinois. Since the nature of its development in the tissues is in some measure similar to that of the Botrytis neck rots, the methods usually practiced for the control of the latter apply equally well to the former. Thorough curing in the field after harvesting results in partial drying of the affected scales, and the spread of the disease in them is thus to a great extent checked. On their removal to the storehouse Walker (*34*) recommends that the onions be dried artificially, especially if moist conditions have prevailed during harvesting and curing. This measure will probably also be beneficial in reducing the amount of rot due to *Macrosporium porri*. After drying, storage in a well-ventilated storehouse kept at temperatures between 33° and 36° F. is essential for securing good keeping quality throughout the storage period.

No experiments for the control of the disease on the leaves and seed stalks have yet been tried.

SUMMARY

Purple blotch of onion caused by *Macrosporium porri* occurs in many of the onion-growing sections of the United States, Cuba, Porto Rico, and Java.

On purple-blotch lesions on the leaves and seed stalks the saprophytic fungus hitherto known as *Macrosporium parasiticum* is often found. For reasons given in the text, this organism should more properly be referred to as *Thyrospora parasitica* (Thüm.) nov. comb.

During the progress of the studies reported in this paper *Macrosporium porri* and *M. solani* have been compared and found to be indistinguishable in their general appearance in culture, in their morphology, and in their physiology. The only essential difference lies in their pathogenicity. Cross-inoculation experiments yielded negative results.

This paper deals more particularly with the morphology, physiology, and pathogenicity of *Macrosporium porri*. Some attention has been been given to its relation to the host and its overwintering in pure culture. The factors influencing the production of coloring matter in artificial media have also been studied. Attempts to isolate the pigments have yielded two, one ether soluble and the other alcohol soluble.

The relative merits of the two generic names Macrosporium and Alternaria have been investigated. Both can be applied to fungi bearing obclavate conidia, but Macrosporium has priority, and therefore its continued use is recommended.

LITERATURE CITED

(1) Berkeley, M. J.
1838. notices of british fungi. Ann. Nat. Hist. 1:257–264, illus.

(2) Bolle, P. C.
1924. die durch schwärzpilze (phaeodictyae) erzeugten pflanzenkrankheiten. Meded. Phytopath. Lab. "Willie Commelin Scholten" 7, 77 p., illus.

(3) Bruner, S. C.
1920. lista preliminar de las enfermedades de las plantas de importancia economica para cuba. Informe Estac. Expt. Agron. Cuba 1918/20: 723–775, illus.

(4) Chapman, G. H.
1910. notes on the occurrence of fungous spores on onion seed. Mass. Agr. Expt. Sta. Ann. Rpt. 22 (1):164–167.

(5) Clinton, G. P.
1904. report of the botanist. Conn. State Agr. Expt. Sta. Ann. Rpt. (1903) 27:279–370, illus.

(6) Cooke, M. C.
1888. new british fungi. Grevillea 16:77–81.

(7) ——— and Ellis, J. B.
1879. new jersey fungi. Grevillea 8:11–16.

(8) Dickson, B. T.
1920. the differential staining of plant pathogen and host. Science (n. s.) 52:63–64.

(9) Edgerton, C. W.
1921. onion diseases and onion seed production. La. Agr. Expt. Sta. Bul. 182, 20 p., illus.

(10) Elliott, J. A.
1917. taxonomic characters of the genera alternaria and macrosporium. Amer. Jour. Bot. 4:439–476, illus.

(11) Fries, E.
1832. systema mycologicum. v. 3. Gryphiswaldae.

(12) ———
[1846]. summa vegetabilium scandinaviae. sectio prior. 572 p. Holmiae and Lipsiae.

(13) Hanna, W. F.
1924. the dry-needle method of making monosporous cultures of hymenomycetes and other fungi. Ann. Bot. [London] 38: 791–795, illus.

(14) Jones, L. R., and Grout, A. J.
1897. notes on two species of alternaria. Bul. Torrey Bot. Club 24:254–258, illus.

(15) Keitt, G. W.
1915. simple technique for isolating single-spore strains of certain types of fungi. Phytopathology 5:266–269, illus.

(16) Kunkel, L. O.
1918. a method of obtaining abundant sporulation in cultures of macrosporium solani E. & M. Brooklyn Bot. Gard. Mem. 1: 306–312, illus.

(17) Miyabe, K.
1889. on the life-history of macrosporium parasiticum, thüm. Ann. Bot. [London] 3:1–26, illus.

(18) NEES VON ESENBECK, C. G.
1816–17. DAS SYSTEM DER PILZE UND SCHWÄMME. 329 p., and atlas. Würzburg.
(19) NOLLA, J. A. B.
1927. A NEW ALTERNARIA DISEASE OF ONIONS (ALLIUM CEPA L.). Phytopathology 17: 115–132, illus.
(20) ORTON, W. A.
1899. A SECOND PARTIAL LIST OF THE PARASITIC FUNGI OF VERMONT. Vt. Agr. Expt. Sta. Ann. Rpt. (1898/99) 12: 164–182.
(21) RANDS, R. D.
1917. EARLY BLIGHT OF POTATO AND RELATED PLANTS. Wis. Agr. Expt. Sta. Research Bul. 42, 48 p., illus.
(22) ———
1917. THE PRODUCTION OF SPORES BY ALTERNARIA SOLANI IN PURE CULTURE. Phytopathology 7: 316–317, illus.
(23) ———
1917. ALTERNARIA ON DATURA AND POTATO. Phytopathology 7: [327]–338, illus.
(24) ROSENBAUM, J.
1920. A MACROSPORIUM FOOT-ROT OF TOMATO. Phytopathology 10: 415–422, illus.
(25) SACCARDO, P. A.
1886–1913. SYLLOGE FUNGORUM. v. 4, 1886; v. 10, 1892; v. 11, 1895; v. 14, 1899; v. 16, 1902; v. 18, 1906; v. 22, 1913. Patavii.
(26) SELBY, A. D.
1910. A BRIEF HANDBOOK OF THE DISEASES OF CULTIVATED PLANTS IN OHIO. Ohio Agr. Expt. Sta. Bul. 214, p. 307–456, illus.
(27) SHIPLEY, A. E.
1889. ON MACROSPORIUM PARASITICUM. Ann. Bot. [London] 3: 268–271.
(28) STEVENSON, J. A.
1918. A CHECK LIST OF PORTO RICAN FUNGI AND A HOST INDEX. Jour. Dept. Agr. Porto Rico 2: 125–264.
(29) TEHON, L. R., and DANIELS, E.
1925. A NOTE ON THE BROWN LEAF-SPOT OF ALFALFA. Phytopathology 15: [714]–719, illus.
(30) TEODORO, N. G.
1923. A STUDY OF A MACROSPORIUM DISEASE OF ONIONS. Philippine Agr. Rev. 16: 233-280, illus.
(31) THAXTER, R.
1890. REPORT OF ROLAND THAXTER, MYCOLOGIST. Conn. State Agr. Expt. Sta. Ann. Rpt. 1889: 127–177, illus.
(32) WALKER, J. C.
1921. A MACROSPORIUM ROT OF ONION. (Abstract) Phytopathology 11: 53.
(33) ———
1921. ONION SMUDGE. Jour. Agr. Research 20: 685–722, illus.
(34) ———
1925. CONTROL OF MYCELIAL NECK ROT OF ONION BY ARTIFICIAL CURING. Jour. Agr. Research 30: 365–373, illus.
(35) WESTENDORP, G.-D.
1863. NOTICE SUR QUELQUES ESPÈCES NOUVELLES OU INÉDITES POUR LA FLORE BELGE. Bul. Soc. Roy. Bot. Belg. 2: 240–254, illus.

CHARACTERS OF THE LARVAE AND PUPAE OF CERTAIN FRUIT FLIES[1]

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INTRODUCTION

The larvae of certain two-winged flies belonging to the dipterous family Trypetidae live within the pulp of fruits and produce adults which, because of the habits of the larvae, are called fruit flies. Some of these species infest fruits of commercial value and cause considerable damage. The Mediterranean fruit fly, the olive fly, and the Mexican fruit fly are excellent examples of injurious species. Some of the fruit flies of other countries are so injurious that quarantines have been established to prevent the entry of these pests into the United States. Other species, such as the apple maggot, and cherry maggot, are native and cause considerable loss to the fruit industries of our country. The forms which occur in foreign countries are often intercepted by quarantine inspectors at various ports of entry into the United States, and this paper should aid these inspectors to recognize the injurious forms which they intercept. The adults of these fruit flies are intercepted only on rare occasions. The larvae of many of the fruit flies have been known to entomologists for many years but the differences between those of the various species have never been tabulated. It is the purpose of this paper to give the distinguishing characters for some of the more important native and exotic species. The flies themselves are easily distinguished by differences in the wing pattern. These differences are shown in Figure 6, but are not discussed in the text. *Toxotrypana curvicauda*, *Anastrepha fraterculus*, *A. striata*, and *A. serpentina* were reared by the writer in Panama in 1926.[2] All of the material discussed in this paper is in the United States National Museum collection. Drawings for all figures illustrating this paper were made by the author.

DEFINITION OF TERMS USED

The structures which constitute some of the principal characters in the accompanying descriptions may be defined as follows:

Spiracular plate (fig. 4, B, *v*): A sclerite containing the spiracular entrances and the button.

Spiracular entrance (fig. 1, A, *a*): One of the passages leading into the main spiracular trachea. This entrance is composed of microscopic perforations.

Peritreme (fig. 1, B, *c*): The chitinous ridge or elevation which directly surrounds and limits a spiracular entrance.

Button (fig. 1, A, *a*): A chitinous area or scar marking the place where the trachea opens on the surface of the first-stage larva.

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[2] The writer is indebted to the personnel of the field laboratory of the Bureau of Entomology in the Canal Zone for facilities and assistance during his stay there in 1926.

Tubules (fig. 1, A): The small projected ends of the anterior spiracle (tubules generally beadlike) arranged in an irregular row.

Mouth hook (fig. 3, A): The mandible or distal part of the cephalopharyngeal skeleton. The latter is composed of three chitinous parts joined together (fig. 3, B, *1*, *2*, *3*); the first is paired and serves for cutting or tearing fruit tissue, its base serving for muscle attachment; the second and third parts serve entirely for muscle attachments. All three parts as an organ are retractile but only the first, or hook, is ever extended outside of the larva.

Fusiform area (fig. 1, A): An integumental part characterized by rows of microscopic chitinous spines hooked at their apices. These spines are arranged somewhat in transverse rows and are used for locomotion.

Anal elevation (fig. 1, A): The circular elevated area in the larvae of certain species, having in its center the anal opening.

Anal lobe (fig. 1, A): A rounded, fleshy, paired organ, usually visible, but sometimes retracted or drawn inside the anal opening.

KEY TO LARVAE OF THE 10 SPECIES CONSIDERED

1. Larva with caudal tubercles[1] ..... 3.
   Larva without caudal tubercles ..... 2.
2. Larva robust; entrances of posterior spiracles large and broad, as in Figure 1, A, *a*; anal lobes protruding, rather small; mouth hooks as in Figure 3, A ..... *Toxotrypana curvicauda* Gerstaecker.
   Larva more slender; entrances of posterior spiracles as in Figure 1, B, *c*; anal lobes not protruding; posterior end with a short transverse line at apex; mouth hooks as in Figure 3, B ..... *Bactrocera cucurbitae* Coquillett.
3. With a very large tubercle on each side of lower half of posterior end and a small tubercle on each side above the posterior spiracles; spiracles as in Figure 1, C, *e*; mouth hooks as in Figure 3, C.
   Ceratitis capitata Wiedemann.
   Without the very large tubercles ..... 4.
4. With two pairs of small caudal tubercles ..... 5.
   With more than two pairs of small caudal tubercles ..... 6.
5. With one small tubercle above and a larger one ventrolaterad of each posterior spiracle; posterior spiracles as in Figure 1, D, *g*; mouth hooks as in Figure 3, D ..... *Dacus oleae* Rossi.
   With one small tubercle above and one directly below each posterior spiracle; posterior spiracles as in Figure 1, E, *i*; mouth hooks as in Figure 3, E.
   Rhagoletis cingulata Loew.
6. With a bifid tubercle below each of the posterior spiracles; posterior spiracles as in Figure 2, A, *k*; mouth hooks as in Figure 3, F.
   Rhagoletis pomonella Walsh.
   Without bifid tubercles ..... 7.
7. Anal lobes bifid; a pair of small tubercles above and below each posterior spiracle; posterior spiracles as in Figure 2, B, *m*; mouth hooks as in Figure 3, H ..... *Anastrepha ludens* Loew.
   Anal lobes entire, not bifid ..... 8.
8. Posterior end with one small tubercle below and two above each spiracle; posterior spiracles as in Figure 2, C, *o*; mouth hooks as in Figure 3, G.
   Anastrepha fraterculus Wiedemann.
   Posterior end not as above ..... 9.
9. Tubercles above the posterior spiracles arranged in a single transverse row; posterior spiracles as in Figure 2, D, *q*; mouth hooks as in Figure 3, I.
   Anastrepha striata Schiner.
   Tubercles above the posterior spiracles arranged in two rows; posterior spiracles as in Figure 2, E, *s*; mouth hooks as in Figure 3, J.
   Anastrepha serpentina Wiedemann.

KEY TO PUPAE OF THE 10 SPECIES CONSIDERED

1. Posterior end with a flat piece projecting down between the spiracular plates. (Fig. 5, D, *cc*.) ..... *Anastrepha striata* Schiner.
   Posterior end without this projecting piece ..... 2.
2. Button large and prominent ..... 3.
   Button small, not so prominent ..... 4.

[1] These structures are often difficult to see. To get the best results, place the larva head downward and remove the surplus fluid with a piece of blotting paper. The tubercles will then be quite evident.

3. Spiracular entrances very broad, two upper ones parallel. (Fig. 5, B, *aa*.)
Anastrepha ludens Loew.
Spiracular entrances narrow, all three radiating, (Fig. 4, A, *u*.)
Toxotrypana curvicauda Gerstaecker.
4. Button indistinct or absent ..... 5.
Button distinct ..... 7.
5. A dark spot (often heart-shaped) at the distal extremity of each spiracular entrance. (Fig. 5, A, *z*.) ..... *Rhagoletis pomonella* Walsh.
Spiracular entrances without the dark spot at the distal extremity ..... 6.
6. Button present; but feebly defined. (Fig. 5, C, *bb*.)
Anastrepha fraterculus Wiedemann.
Button absent. (Fig. 5, E, *dd*.) ..... *Anastrepha serpentina* Wiedemann.
7. Button apparently detached from the spiracular plate. (Fig. 4, B, *v*.)
Bactrocera cucurbitae Coquillett.
Button obviously attached to spiracular plate ..... 8.
8. Spiracular plate slightly crescent shaped ..... 9.
Spiracular plate not crescent shaped, about as broad as high; spiracular entrances narrow; button at the upper inner portion. (Fig. 4, E, *y*.)
Rhagoletis cingulata Loew.
9. Spiracular entrances very broad and long, plate large. (Fig. 4, D, *x*.)
Dacus oleae Rossi.
Spiracular entrances narrow and short, plate small (Fig. 4, C, *w*.)
Ceratitis capitata Wiedemann.

DESCRIPTIONS

TOXOTRYPANA CURVICAUDA Gerstaecker

(Papaya fruit fly; wing, fig. 6, A)

Larva (fig. 1, A).—Pale yellowish white, cylindrical, tapering slightly toward cephalic end; 11 segments of about equal length in addition to the head; a ventral fusiform area on anterior portion of each of segments 4 to 11. Head (fig. 1, A, *b*) small, partly retractile; each side of front bearing two very small, pale yellowish-brown, chitinized tubercles, the antenna (fig. 1, A, *b*) slightly larger and apparently having a constriction in the middle; mouth hooks (fig. 3, A) fairly large, hooks robust, first and second parts black, third part with a deep brown infuscation fading to hyaline at apices. Anterior spiracles small, yellow, chitinized, and with from 25 to 28 small rounded tubules in an irregular row. Posterior spiracles (fig. 1, A, *a*) large, located far above the medio-horizontal line; each spiracle with three broad yellowish entrances, each with a dark brown peritreme; button round, weak, and sometimes wanting; inner ends of the bottom entrances of the spiracles separated by a space about one and one-fourth times the length of one entrance; sometimes a broad, crescent-shaped band of yellow chitin showing beneath the surface upon which the spiracular entrances are located. Anal elevation large, with two small rounded anal lobes.

Length 13 mm.; diameter 2 mm.

Described from 35 larvae from material collected by C. T. Greene and I. Molino and reared by C. T. Greene at Ancon, Canal Zone, March 20 to April 30, 1926.

Host.—Papaya fruit.

Pupa (fig. 4, A).—Cylindrical, dull luteous to deep reddish brown; 11 distinct segments, the last vertically constricted with its lower half prominently projecting. Anterior spiracles like those of the larva but slightly darker. Posterior spiracles of medium size (fig. 4, A, *u*), deep reddish brown, located above the medio-horizontal line in a faintly depressed triangle which has a small rounded depression at each

basal angle; each spiracular entrance yellowish, located on top of a well-defined ridge; button large, pale yellow, circular, with a small depression in the center. Anal opening small; anal plates large, forming an elliptical dark-red area.

Length 9 to 10 mm.; diameter 3 to 3.75 mm.

Described from 25 specimens from the same source as the larvae.

BACTROCERA CUCURBITAE Coquillett

(Melon fly; wing, fig. 6, C)

Larva (fig. 1, B).—Pale yellowish white, cylindrical, tapering slightly toward cephalic end; 11 segments of about equal length in addition to the head; eleventh segment somewhat dome-shaped,with a short transverse black line at apex; a ventral fusiform area on anterior portion of each of segments 2 to 11; a dorsal fusiform area on anterior portion of each of segments 1 to 3. Head (fig. 1, B, *d*) small, partly retractile; each side of front bearing two very small, pale yellowish-brown, chitinized tubercles, the antenna (fig. 1, B, *d*) more prominent; mouth hooks (fig. 3, B) large, hooks long and slender, first and second parts black, third part with a deep brown infuscation fading to hyaline at apices. Anterior spiracles small, yellow, chitinized, and with from 17 to 19 small rounded tubules in an irregular row. Posterior spiracles (fig. 1, B, *c*) medium sized, located well above the medio-horizontal line; each spiracle with three broad yellowish entrances, each with a dark-brown peritreme; button small, round, weak, and sometimes wanting; inner ends of the bottom entrances of the spiracles separated by a space about one and one-third times the length of one entrance. Anal opening located in an elliptical depression; anal lobes small and round; posterior to each end of the anal depression a small elliptical depression. (Fig. 1, B.)

Length 8 to 10.5 mm.; diameter 1.75 to 2.75 mm.

Described from numerous specimens collected at Honolulu, Hawaii, August 5, 1926.

Hosts.—Cantaloupe (rock melon), cucumber, bitter gourd, eggplant, and sometimes watermelon.

Pupa (fig. 4, B).—Cylindrical, dull luteous to deep reddish yellow; 11 distinct segments, the last segment a little more prominent on the lower half. Anterior spiracles like those of the larva but much darker. Posterior spiracles (fig. 4, B, *v*) medium sized, deep yellowish brown, located partially on but mostly below medio-horizontal line; each spiracle having three small fairly broad entrances each of which has the immediate surrounding area paler; button small, round, and appearing to be separated from the plate. Anal plates large, round, and deep red.

Length 4 to 6 mm.; diameter 1.7 to 2.5 mm.

Described from numerous specimens from the same source as the larvae.

CERATITIS CAPITATA Wiedemann

(Mediterranean fruit fly; wing, fig. 6, B.)

Larva (fig. 1, C).—Pale yellowish white, cylindrical, tapering slightly toward cephalic end; 11 segments of about equal length in addition to the head; a ventral fusiform area on anterior portion of each of segments 2 to 11; a dorsal fusiform area on anterior portion of each of segments 1 to 3. Head (fig. 1, C, *f*) small, partly retractile;

each side of the front bearing two small tubercles, the antenna (fig. 1, C, *f*) pale yellowish brown, and chitinized; mouth hooks (fig. 3, C) small, hooks slender, first and second parts black, third part with dark-brown infuscation fading to hyaline at apices. Anterior spiracles small, yellow, chitinized and with from 10 to 14 small rounded tubules in an irregular row. Posterior spiracles (fig. 1, C, *e*) o :medium size, located some distance above the medio-horizontal line; each spiracle with three broad yellow entrances, each with a much darker peritreme; button small, round, whitish, and not distinctly visible; inner ends of the bottom entrances of the spiracles separated by a space about equal to the length of one entrance. Above each posterior spiracle a small tubercle, and below each spiracle one very large tubercle. Anal elevation large; anal lobes large and rounded.

Length 7 to 9 mm.; diameter 1 to 2 mm.

Described from 50 larvae collected at Honolulu, Hawaii, August 10, 1926.

Hosts.—Grape, peach, mango, apricot, pear, plum, apple, fig, kei apple (*Aberia caffra*), orange, lemon, pompelmoes, *Citrus buxifolius*, passion flower (*Passiflora caerulea*), nectarine, quince, kafir plum (*Harpephyllum caffrum*), *Solanum capsicastrum*, loquat, guava, and prickly pear.

Pupa (fig. 4, C).—Cylindrical, pale luteous to deep reddish brown; 11 distinct segments. Anterior spiracles like those of the larva but darker. Posterior spiracles (fig. 4, C, *w*) of medium size, yellowish red to dark red, located centrally on medio-horizontal line; each spiracle with three small yellowish entrances located on a crescent-shaped plate; button small, rounded, and not very distinct. Anal plates small, very dark red.

Length 3 to 5 mm.; diameter 1.25 to 2 mm.

Described from numerous specimens from the same source as the larvae.

DACUS OLEAE Rossi

(Olive fruit fly; wing, fig. 6, D)

Larva (figure. 1, D).—Yellowish white, tapering slightly toward cephalic end; 11 segments of equal length in addition to the head; a ventral fusiform area on anterior portion of each of segments 2 to 11. Head (fig. 1, D, *h*) small, partly retractile; each side of front bearing two small tubercles, the antenna (fig. 1, D, *h*) heavily chitinized; mouth hooks (fig. 3, D) small, hooks robust, first and second parts black, third part with dark-brown infuscation fading to hyaline at apices. Anterior spiracles small, yellow, chitinized, with from seven to nine small rounded tubules in a row. Posterior spiracles (fig. 1, D, *g*) small, located well above the medio-horizontal line; each spiracle with three short, broad, yellow entrances, each with a dark-brown peritreme; button rounded and indistinct; inner ends of the bottom entrances of the spiracles separated by a space about two and one-half times the length of one entrance; a little above and to the outside of each posterior spiracle a small tubercle, and at the extreme ends of the medio-horizontal line a large, somewhat rounded tubercle. No anal elevation; anal opening very narrow.

Length 5 to 6 mm.; diameter 1 to 1.25 mm.

Described from 31 larvae. In olives from Italy, intercepted at Philadelphia, Pa., April 5, 1926, by Max Kisliuk.

Host.—Olive.

Pupa (fig. 4, D).—Cylindrical, pale luteous; 11 distinct segments. Anterior spiracles like those of the larvae but a little darker. Posterior spiracles (fig. 4, D, *x*) small, located centrally on mediohorizontal line at apex; each spiracle with three short, broad, yellowish entrances; plate crescent shaped, with a lighter-colored area on inner side; button small, rounded, and not very distinct. Anal plates small, round, dark reddish.

Length 4 mm.; diameter 2 mm.

Described from five specimens from the same source as the larvae.

RHAGOLETIS CINGULATA Loew

(Cherry maggot; wing, fig. 6, E)

Larva (fig. 1, E).—Pale yellowish white, cylindrical, tapering slightly toward cephalic end; 11 segments of about equal length in addition to the head; a ventral fusiform area on anterior portion of each of segments 4 to 11. Head (fig. 1, E, *j*) small, partly retractile; each side of front bearing two small, rounded tubercles, the antenna (fig. 1, E, *j*) slightly larger and chitinized; mouth hooks (fig. 3, E) medium sized, hooks fairly long and slender, first and second parts black, third part with a deep-brown infuscation fading to hyaline at apices. Anterior spiracles small, yellow, chitinized, and with from 22 to 25 small rounded tubules arranged in two irregular rows. Posterior spiracles (fig. 1, E, *i*) small, located considerably above the mediohorizontal line; each spiracle with three broad yellow entrances, each with a dark yellowish brown peritreme; button small, weakly defined; inner ends of the lower spiracular entrances of the spiracles separated by a space equal to one and one-half times the length of one entrance; above each posterior spiracle a small tubercle, and below each spiracle a larger tubercle. Anal elevation large, with two rounded, tuberculate anal lobes.

Length 7.5 to 8 mm.; diameter 1.5 mm.

Described from 10 specimens collected at Ithaca, N. Y., July, 1912, by J. F. Illingworth.

Hosts.—Cherries (sour and sweet).

Pupa (fig. 4, E).—Small, cylindrical, dull luteous; 11 distinct segments. Anterior spiracles like those of the larva but slightly darker. Posterior spiracles (fig. 4, E, *y*) medium sized, reddish brown, located on upper side of medio-horizontal line; each spiracle with three narrow yellow entrances; button small, located on upper part of inner side of plate. Anal plate medium sized, round and deep reddish yellow.

Length 3 to 4 mm.; diameter 1.5 to 1.75 mm.

Described from 10 specimens from the same source as the larvae.

RHAGOLETIS POMONELLA Walsh

(Apple maggot; wing, fig. 6, F)

Larva (fig. 2, A).—Pale yellowish white, cylindrical, tapering slightly toward cephalic end; 11 segments of about equal length in addition to the head; a definite ventral fusiform area on anterior portion of each of segments 4 to 11. Head (fig. 2, A, *l*) small, partly retractile; each side of front bearing two small, broadly rounded tubercles; the antenna (fig. 2, A, *l*); mouth hooks (fig. 3, F) small, robust, first and second parts black, third part with brown infuscation

at the base fading to hyaline at apices. Anterior spiracles small, yellow, chitinized, and with from 15 to 17 small rounded tubules in an irregular row. Posterior spiracles (fig. 2, A, *k*) small, located considerably above the medio-horizontal line; each spiracle with three narrow yellowish entrances, each with a slightly darker peritreme; button small, not very distinct; inner ends of the bottom entrances of the spiracles separated by a space about one and one-half times the length of one entrance; above each posterior spiracle a small tubercle and ventro-laterad two more small tubercles; in the middle two bifid tubercles widely separated; a small tubercle on each side near base of anal elevation. Anal elevation large, with the lobes tuberculate and rounded.

Length 7.25 to 8 mm.; diameter 1.5 mm.

Described from 20 larvae collected at Ithaca, N. Y., July, 1911, by J. F. Illingworth.

Hosts.—Apple, fruit of several species of Crataegus, huckleberry, blueberry, snowberry, prune, and cranberry.

Pupa (fig. 5, A).—Cylindrical, dull luteous; 11 distinct segments, the last projecting slightly on the lower half. Anterior spiracles like those of the larva but slightly darker. Posterior spiracles (fig. 5, A, *z*) small, reddish yellow, located slightly above the medio-horizontal line; each spiracle with three narrow entrances; at the outer end of each entrance a small, dark-brown, heart-shaped spot; button apparently wanting. Anal plates small, dark reddish brown.

Length 4 to 5 mm.; diameter 1.5 to 2 mm.

Described from 18 specimens from the same source as the larvae.

ANASTREPHA LUDENS Loew

(Mexican fruit worm; wing, fig. 6, G)

Larva (fig. 2, B).—Pale yellowish white, cylindrical, tapering gradually toward cephalic end; 11 segments of about equal length in addition to the head; a ventral fusiform area on the anterior portion of each of segments 2 to 11. Head (fig. 2, B, *n*) small, partly retractile; each side of front bearing two small tubercles, the antenna (fig. 2, B, *n*) larger and having a yellowish chitinized thimblelike projection at apex; mouth hooks (fig. 3, H) large, hooks robust, first and second parts black, third part with a dark-brown infuscation at base and gradually clearing to hyaline at apices. Anterior spiracles small, pale yellow, chitinized, and with 13 to 14 small rounded tubules in an irregular row. Posterior spiracles (fig. 2, B, *m*) small, located well above the medio-horizontal line; each spiracle with three broad yellow entrances, each with a dark-brown peritreme; button round and indistinct; inner ends of bottom entrances of the spiracles separated by a space about one and one-half times the length of one entrance; above and below each posterior spiracle a pair of small tubercles. Anal elevation large, with each anal lobe large, bifid, and with a dark-brown infuscation.

Length 9 to 10 mm.; diameter 1.5 mm.

Described from 18 larvae. From Mexico, intercepted at El Paso, Tex., August 11, 1924, by T. A. Arnold, collector. El Paso No. 147.

Hosts.—Orange, grapefruit, sweet lime, mango, *Sideroxylon sapota*, peach, plum, and guava.

Pupa (fig. 5, B).—Cylindrical, pale luteous to dark red; 11 segments, the last slightly prominent on the lower half. Anterior spiracles like

those of the larva but darker. Posterior spiracles (fig. 5, B, *aa*) of medium size, deep reddish brown, located on but mostly below the medio-horizontal line; each spiracle with three broad yellow entrances mounted on top of well-defined ridges; button large, yellowish, with a depression in the middle. Anal plates large, elliptical, and reddish black.

Length 5.5 to 7.5 mm.; diameter 2 to 3.25 mm.

Described from 8 specimens from the same source as the larvae.

ANASTREPHA FRATERCULUS Wiedemann

(West Indian fruit fly; wing, fig. 6, H)

Larva (fig, 2, C).—Pale yellowish white, cylindrical, tapering slightly toward cephalic end; 11 segments of about equal length in addition to the head; a ventral fusiform area on anterior portion of each of segments 4 to 11. Head (fig. 2, C, *p*) small, partly retractile; each side of front bearing two small tubercles, the antenna (fig. 2, C, *p*) more chitinized and projecting thimblelike; mouth hooks (fig. 3, G) medium sized, first part rather slender, first and second parts black, third part with a brown infuscation at base and the rest of the joint hyaline. Anterior spiracles small, yellowish, chitinized, and with from 15 to 17 small rounded tubules arranged in an irregular row. Posterior spiracles (fig. 2, C, *o*) small, located well above the medio-horizontal line; each spiracle with three broad yellow entrances, each with a dark-brown peritreme; button large but indistinct; inner ends of the bottom entrances of the spiracles separated by a space about one and one-fourth times the length of one entrance; above each posterior spiracle two small tubercles close together and one tubercle below each spiracle. Anal elevation large, with two rounded lobes.

Length 8 to 10 mm.; diameter 1.5 mm.

Described from 10 larvae from material collected by C. T. Greene and I. Molino and reared by C. T. Greene from red plum, Panama City, Panama, May 3 to 15, 1926.

Hosts.—Orange, *Spondias mombin*, *Spondias lutea*, guava (*Psidium guajava*), coffee berries, pear, peach, mango, grape, Phylocalyx, Japanese plum, Japanese persimmon, Para plum (Spondias spp.), *Annona humboldtiana*, jobo amarillo, jobo de la India, tangerine, Cuban plum sapodilla, and *Eugenia malaccensis*.

Pupa (fig. 5, C).—Cylindrical, dull luteous to reddish yellow or dark red; 11 distinct segments. Anterior spiracles like those of the larva but much darker. Posterior spiracles (fig. 5, C, *bb*) medium sized, dark reddish, located in a faintly depressed hexagonal area which is on but mostly below the medio-horizontal line; each spiracle with three narrow yellowish entrances, each on top of a well-defined ridge; button small and weakly defined. Anal plates small, rough, and deep red to blackish.

Length 4.5 to 6 mm.; diameter 2 to 2.5 mm.

Described from 20 specimens from the same source as the larvae.

ANASTREPHA STRIATA Schiner

(Central American fruit fly; wing, fig. 6, I)

Larva (fig. 2, D).—Pale yellowish white, cylindrical, tapering slightly toward cephalic end; 11 segments of about equal length in addition to the head; a ventral fusiform area on anterior portion of each of segments 3 to 11. Head (fig. 2, D, *r*) small, partly retractile; each side of front bearing two broadly rounded, somewhat flattened tubercles, the antenna (fig. 2, D, *r*) larger and having a small rounded nipplelike tubercle at apex; mouth hooks (fig. 3, I) large, first part large, first and second parts black, third part with a dark-brown infuscated area at base, the rest of the joint hyaline. Anterior spiracles small, with from 16 to 17 small rounded tubules in an irregular row. Posterior spiracles (fig. 2, D, *q*) medium sized, located considerably above the medio-horizontal line; each spiracle with three broad, long, yellow entrances, each with a dark-brown peritreme; button large, round, and indistinct; inner ends of the bottom entrances of the spiracles separated by a space about equal to the length of one entrance; above each spiracle two small tubercles close together, and below each spiracle two small tubercles much wider apart. Anal elevation large, rounded, and with two prominent rounded lobes.

Length 7 to 9 mm.; diameter 1.5 mm.

Described from 11 larvae from material collected by C. T. Greene and I. Molino and reared by C. T. Greene from guava taken at La Sabanas, Panama, April 5 to May 9, 1926, and Frijoles, Canal Zone, April 12, 1926.

Host.—Guava.

Pupa (fig. 5, D).—Cylindrical, dull luteous to reddish yellow; 11 distinct segments. Anterior spiracles like those of the larva but darker. Between the posterior spiracles a broad, flattened projection extending down between the upper ends of the spiracular plates; posterior spiracles (fig. 5, D, *cc*) medium sized, dark reddish, located in a faintly depressed hexagonal area which is on but mostly above the medio-horizontal line; each spiracle with three broad yellow entrances, each located on a well defined ridge; button small and indistinct. Anal plates small, deep reddish.

Length 4.5 to 5.5 mm.; diameter 2 to 2.5 mm.

Described from 74 specimens from the same source as the larvae.

ANASTREPHA SERPENTINA Wiedemann

(Dark fruit fly; wing, fig. 6, J)

Larva (fig. 2, E.)—Pale yellowish white, tapering slightly toward cephalic end; 11 segments of equal length in addition to the head; a ventral fusiform area on each of segments 4 to 11. Head (fig. 2, E, *t*) small, partly retractile; each side of front bearing two small tubercles, the antenna (fig. 2, E, *t*) larger, pale yellow, and chitinized; mouth hooks (fig. 3, J) large, hooks robust, first and second parts black, third part with a dark-brown infuscation at base fading gradually to hyaline at apices. Anterior spiracles small, yellow, chitinized, and with from 16 to 18 small rounded tubules in an irregular row. Posterior spiracles (fig. 2, E, *s*) medium sized, located considerably above the medio-horizontal line; each spiracle with three broad yellow entrances, each with a dark-brown peritreme; button round and indistinct; inner ends of the bottom entrances of the spiracles

separated by a space about one and two-thirds times the length of one entrance; above each posterior spiracle two small tubercles, one above the other, and below each spiracle two small tubercles in a transverse line, widely separated. Anal elevation large, with two medium-sized rounded lobes.

Length 9 to 10 mm.; diameter 1.5 mm.

Described from 16 larvae from material collected by C. T. Greene and I. Molino at La Sabanas and Panama City, Panama, and Ancon, Canal Zone, March 17, 1926.

Hosts—Nispero, star apple, guava, and various Sapotaceae.

Pupa (fig. 5, E.)—Cylindrical, dull pale luteous to reddish; 11 distinct segments. Anterior spiracles like those of the larva but darker. Posterior spiracles (fig. 5, E, *dd*) medium sized, dark reddish, located almost centrally on the medio-horizontal line; each spiracle with three yellowish entrances, each located on a well-defined ridge; button apparently wanting. Anal plates large, dark red, and round.

Length 5 to 5.5 mm.; diameter 2.5 mm.

Described from 106 specimens from the same source as the larvae.

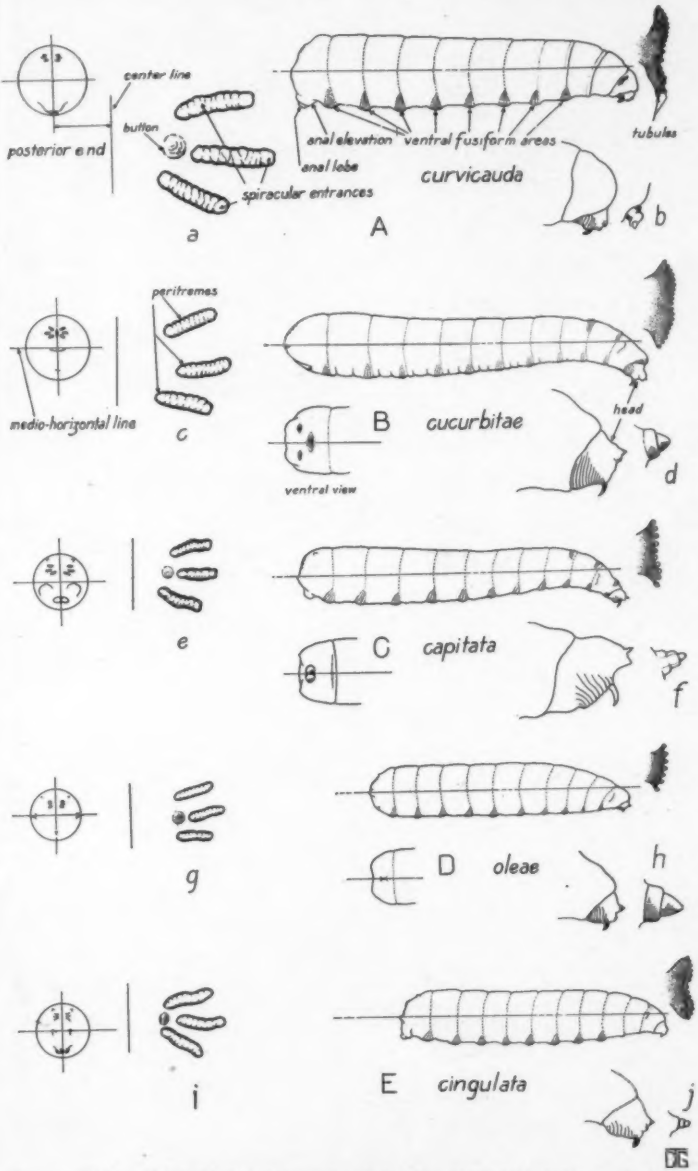

FIGURE 1. Larvae of fruit flies, lateral view and posterior view, × 6; right posterior spiracle, × 50. A.—*Toxotrypana curvicauda: a*, posterior spiracle; *b*, antenna. B.—*Bactrocera cucurbitae: c*, posterior spiracle; *d*, antenna. C.—*Ceratitis capitata: e*, posterior spiracle; *f*, antenna. D.—*Dacus oleae: g*, posterior spiracle; *h*, antenna. E.—*Rhagoletis cingulata: i*, posterior spiracle; *j*, antenna

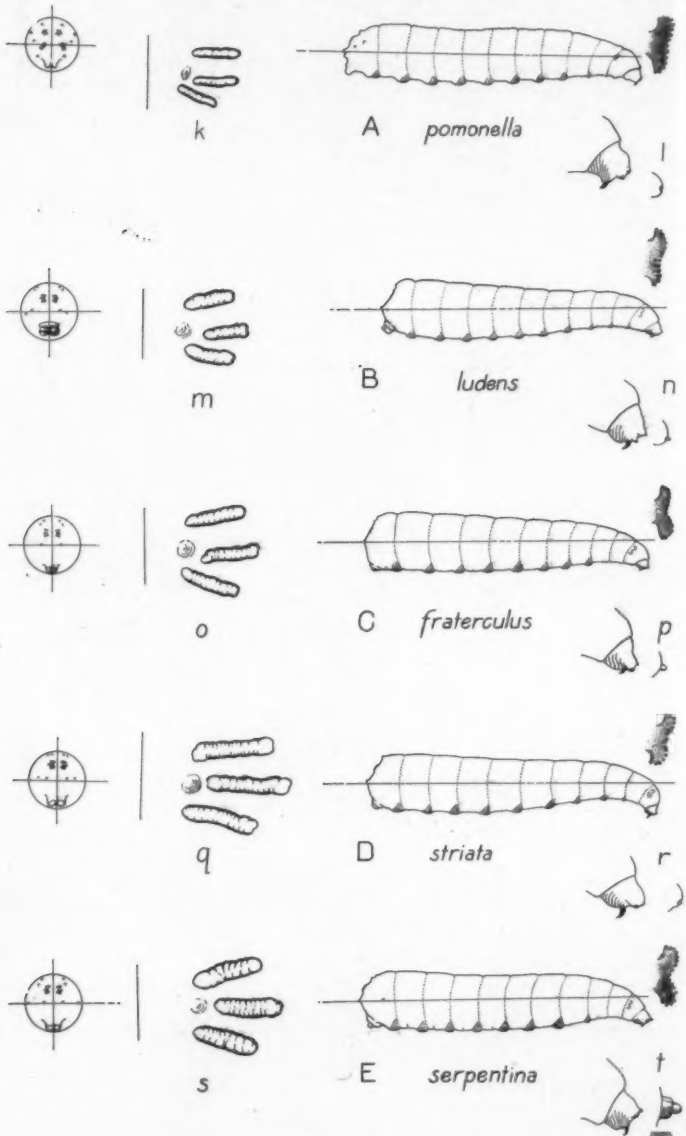

FIGURE 2.—Larvae of fruit flies, lateral view and posterior view, × 6; right posterior spiracle, × 50. A.—*Rhagoletis pomonella: k*, posterior spiracle; *l*, antenna. B.—*Anastrepha ludens: m*, posterior spiracle; *n*, antenna. C.—*A. fraterculus: o*, posterior spiracle; *p*, antenna. D.—*A. striata: q*, posterior spiracle; *r*, antenna. E.—*A. serpentina: s*, posterior spiracle; *t*, antenna

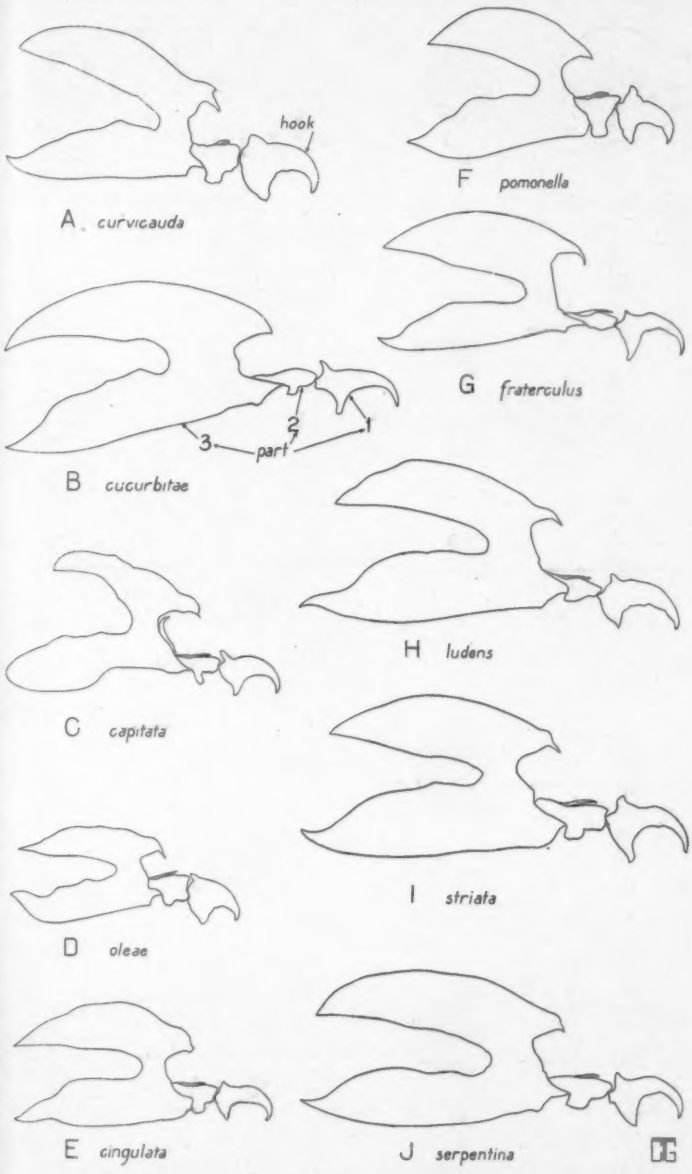

FIGURE 3.—Mouth hooks of larvae of fruit flies, × 50: A, *Tozotrypana curvicauda;* B, *Bactrocera cucurbitae;* C, *Ceratitis capitata;* D, *Dacus oleae;* E, *Rhagoletis cingulata;* F, *R. pomonella;* G, *Anastrepha fraterculus;* H, *A. ludens;* I, *A. striata;* J, *A. serpentina*

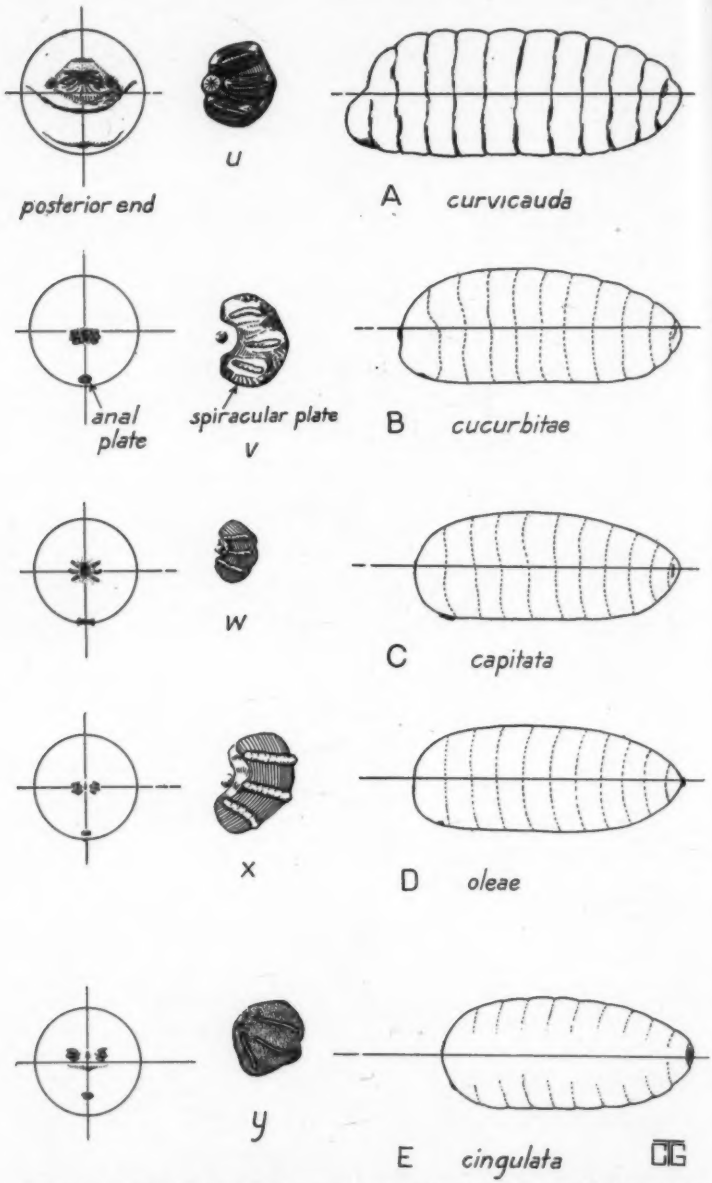

FIGURE 4.—Pupae of fruit flies, lateral view and posterior view, × 6; right posterior spiracle, × 50. A.—*Toxotrypana curvicauda: u*, posterior spiracle. B.—*Bactrocera cucurbitae: v*, posterior spiracle. C.—*Ceratitis capitata: w*, posterior spiracle. D.—*Dacus oleae: x*, posterior spiracle. E.—*Rhagoletis cingulata: y*, posterior spiracle

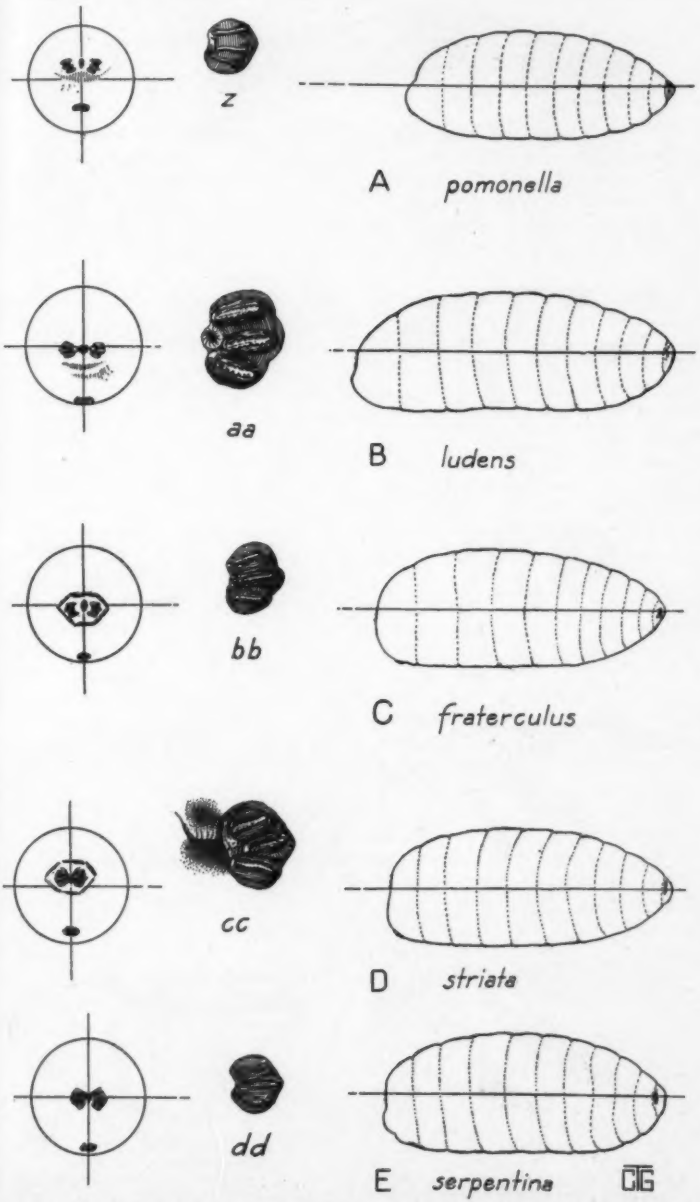

FIGURE 5.—Pupae of fruit flies, lateral view and posterior view, × 6; right posterior spiracle, × 50. A.—*Rhagoletis pomonella: z*, posterior spiracle. B.—*Anastrepha ludens: aa*, posterior spiracle. C.—*A. fraterculus: bb*, posterior spiracle. D.—*A. striata: cc*, posterior spiracle. E.—*A. serpentina: dd*, posterior spiracle

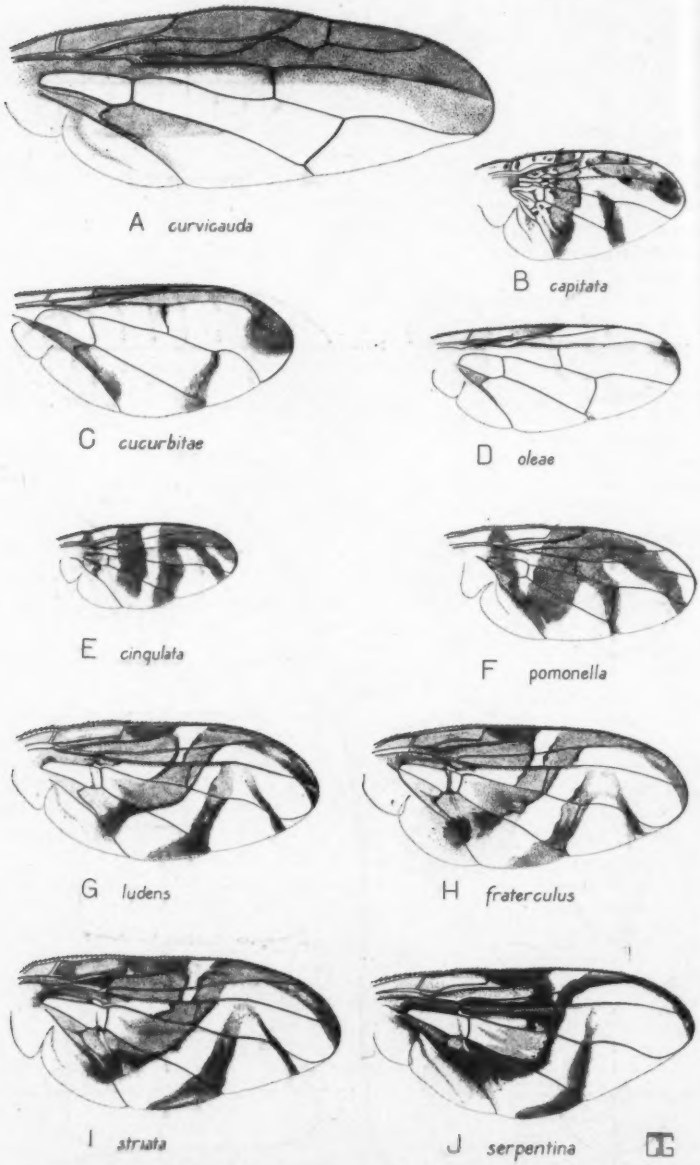

FIGURE 6.—Wings of fruit flies (right wing drawn), ×7.5: A, *Toxotrypana curvicauda;* B, *Ceratitis capitata;* C, *Bactrocera cucurbitae;* D, *Dacus oleae;* E, *Rhagoletis cingulata;* F, *R. pomonella;* G, *Anastrepha ludens;* H, *A. fraterculus;* I, *A. striata;* J, *A. serpentina*

RECESSIVE DEFECTS AND YIELD IN CORN[1]

By JOHN B. WENTZ, *Assistant Chief in Farm Crops, Iowa Agricultural Experiment Station*, and SAMUEL F. GOODSELL,[2] *Student, Iowa State College*

INTRODUCTION

Self-pollination in corn has shown that commercial varieties contain large numbers of recessive lethal and semilethal heritable defects. Breeders have assumed that these defects are detrimental to yield, and have been eliminating them from their selfed lines. It is evident that if appreciable numbers of the defects were present in a variety in the homozygous recessive condition, yield would be affected. It also is logical to assume that recessive defects may have some effect upon yield when in the heterozygous condition, since complete dominance in inheritance is the exception rather than the rule.

A number of investigators have made attempts to determine the effect of heritable abnormalities or defects, when present in the heterozygous condition, upon yields of individual selfed plants or progenies. The results have recently been reviewed by Garber and Rowley (*2*),[3] Wentz and Stewart (*8*) and Mangelsdorf (*6*). In general, the results are not consistent in showing differences in favor of the homozygous normal plants or progenies as compared with those which were heterozygous for the abnormalities. Some of the experiments showed significant differences in favor of the homozygous normal plants; in some the differences were very slight, and in others there were no significant differences.

An attempt was made in the study reported in this paper to see if there is any relation between the number of the heritable abnormalities or defects present in a commercial variety of corn and the yield of that variety. The problem was suggested by the fact that some of our Corn Belt varieties found most productive in yield comparisons, show large numbers of recessive defects when self-pollinated.

Collins (*1*) in comparing 20 lines, each coming from a single ear of the variety Sacaton June, found after self-pollination, a significant negative correlation (-0.48 ± 0.12) between the number of abnormalities appearing and length of ear. If it is assumed that length of ear is a fair measure of yield, the results indicate some relation between the number of abnormalities present and yield in these lines. Kyle and Stoneberg (*3*) report that after selecting for number of rows of kernels in selfed lines, the resulting higher-yielding lines had fewer plants with heritable deleterious characters than the lower-yielding lines. In the experiments of Collins and of Kyle and Stoneberg, both the yield and the notes on abnormalities appearing were taken on the selfed lines. In the present study, yields were determined on the commercial open-pollinated varieties. Seed then was planted from each variety and a large number of plants were selfed. The numbers of abnormalities present in the varieties were determined from these selfed progenies.

[1] Received for publication Jan. 28, 1929; issued May, 1929. Published with the approval of the director of the Iowa Agricultural Experiment Station.

[2] Now research fellow, Department of Field Crops, University of Missouri.

[3] Reference is made by number (italic) to "Literature cited," p. 510.

MATERIAL AND METHODS

The yield data were supplied by J. L. Robinson and A. A. Bryan from the records of the Iowa corn-yield test. In this test the farmer sends to the secretary of the Iowa Corn and Small Grain Growers' Association seed of the variety he wishes to enter in the test. The varieties are then grown under uniform conditions in a section of the State to which they are adapted and yields determined. The yields used in this problem were taken from the Jones County field in the eastern part of the State. It was found that in this field there were 19 varieties which had been entered two consecutive years (1925 and 1926), and that there was considerable range in the yields of the 19 varieties. All of the varieties were representative of the type of corn grown in this section of the Corn Belt. Most of the varieties had been grown for a number of years by the farmers entering them in the test, and some of them had been grown and carefully selected over long periods of years.

In the yield test, ten 3-row plots of each variety, 25 hills long, were systematically distributed over the field. The middle row of each plot was harvested. At harvest, a 100-pound sample of ears was taken from each variety for the determination of the moisture content and of the shelling percentage. The final acre yields were expressed in bushels of dry shelled corn.

In the spring of 1926 some of the remnant seed of each of the 19 varieties was planted in college field at the experiment station and during the summer approximately 50 ears of each variety were self-pollinated. After these selfed ears were harvested and cured, each was shelled and examined for seed defects and then 25 seeds from each ear were planted in the greenhouse and the seedling progenies examined for defective types. In the spring of 1927, 31 seeds from each of the ears were planted in the field to check the greenhouse tests on seedling defects and to get notes on plant defects.

All the defects studied were classified into three groups—seed defects, seedling chlorophyll defects, and plant defects. The seed defects were divided into those affecting the endosperm and those affecting the embryo. The seedling chlorophyll defects are chlorophyll abnormalities which could be identified in the seedling stage in the greenhouse tests and were classified according to their appearance. No attempt was made to determine genetic factors causing the defects in any of the three classes, and since the numbers of seedlings and plants from a single ear were small, no particular emphasis was put on determining Mendelian ratios. The essential point was to determine the ears which were segregating for each of the defects. It was thought that the 25 seeds planted in the greenhouse with the additional 31 seeds planted in the field from each ear would be almost certain to bring out all ordinary seedling defects involving one or two factors. The plant defects were determined from only the 31 seeds planted in the field, so it is possible that a few of the plant defects might have escaped notice, but it is believed that this number is small.

Some difficulty was experienced in determining progenies segregating for barren plants. It was finally decided to classify a progeny (approximately 30 plants from a single ear) as segregating for barren plants when one or more healthy plants were barren. Progenies containing barren plants due to severe infections of smut were not classified as segregating for barren plants.

EXPERIMENTAL DATA

The number of plants selfed in each variety and the percentage of the resulting progenies which showed the various defects are recorded in Table 1. The varieties are listed in the order of their mean yields for the two years in order more easily to identify any correlations between yield and the percentages of progenies segregating for defects. The 19 varieties are divided in the table into three groups of the 6 highest yielders, the 6 lowest yielders and the middle 7. The average percentages of segregating progenies are shown for each group.

The data in Table 1 show no definite relation between numbers of defects present and yield of the varieties. Five of the 10 abnormalities studied showed larger averages in the 6 low-yielding varieties than in the 6 high-yielding varieties. For 1 of these, striped, the difference was outstanding. One of the remaining 5 abnormalities, showing larger averages in the 6 high-yielding than in the 6 low-yielding varieties, there is 1, pale green, in which the difference is outstanding. When the abnormalities under each of the three classes are totaled, there are no significant differences in the numbers found among the high and among the low-yielding varieties. The same is true when all abnormalities are totaled.

TABLE 1.—*Yields of 19 varieties of corn and the percentages of the selfed progenies from them which were segregating for various defects*

Variety No.	Acre yield in bushels in—			Number of ears tested	Percentage of progenies segregating for—													
					Seed defects			Seedling chlorophyll defects						Plant defects				All defects
	1925	1926	Two-year average		Endosperm	Germ	Total	White	Striped	Virescent	Yellow	Pale green	Total	Dwarf	Golden	Barren	Total	
14	101.73	69.27	85.50	60	3.32	3.33	6.65	5.00	4.99	3.33	0.00	5.00	18.32	6.66	1.66	55.00	63.32	88.29
15	91.00	62.52	76.76	51	7.84	.00	7.84	3.92	1.96	.00	1.96	1.96	9.80	1.96	.00	62.75	64.71	82.35
16	87.43	65.02	76.22	58	13.79	3.44	17.23	8.62	1.72	.00	5.17	.00	15.51	5.17	1.72	48.28	55.17	87.91
5	78.03	66.75	72.39	42	14.28	9.52	23.80	16.66	.00	7.14	11.90	7.14	42.84	7.14	.00	78.57	85.71	152.35
9	74.33	69.47	71.90	25	12.00	4.00	16.00	8.00	.00	.00	8.00	12.00	28.00	8.00	.00	80.00	88.00	132.00
4	71.38	70.92	71.15	56	1.78	1.78	3.56	3.57	3.57	.00	3.57	.00	10.71	5.35	.00	87.50	92.85	107.12
Av	83.98	67.32	75.65		8.83	3.68	12.51	7.63	2.04	1.74	5.10	4.35	20.86	5.71	.56	68.68	74.95	108.34
19	70.10	71.88	70.99	55	3.63	5.45	9.08	.00	.00	1.81	1.81	.00	3.62	1.81	1.81	54.55	58.17	70.87
12	73.00	68.10	70.55	54	11.10	5.55	16.65	5.55	1.85	.00	5.55	.00	12.95	1.85	.00	69.23	71.08	100.68
2	74.53	66.37	70.45	40	2.50	5.00	7.50	15.00	10.00	2.50	2.50	2.50	32.50	2.50	.00	57.50	60.00	100.00
1	74.95	65.15	70.05	39	12.81	.00	12.81	10.25	5.12	2.56	2.56	7.69	28.18	2.56	.00	71.79	74.35	115.34
10	77.45	60.53	68.99	58	.00	6.89	6.89	5.17	10.34	3.44	.00	.00	18.95	.00	.00	46.55	46.55	72.39
8	70.68	66.70	68.69	61	1.63	1.63	3.26	3.27	.00	.00	3.27	4.91	11.45	3.27	.00	59.02	62.29	77.00
6	76.83	59.81	68.32	47	6.37	.00	6.37	2.12	.00	2.12	2.12	6.38	12.74	.00	.00	95.56	95.56	114.67
Av	75.36	65.51	69.72		5.43	3.50	8.94	5.91	3.90	1.78	2.54	3.07	17.20	1.71	.26	64.89	66.86	92.99
13	69.78	64.79	67.28	50	4.00	4.00	8.00	6.00	.00	.00	6.00	.00	12.00	6.00	.00	72.00	78.00	98.00
17	70.08	59.36	64.72	52	11.52	7.69	19.21	1.92	3.84	1.92	1.92	3.84	13.44	5.76	.00	80.77	86.53	119.18
7	66.83	60.63	63.73	27	18.51	.00	18.51	7.40	7.40	.00	3.70	.00	18.50	3.70	.00	63.16	66.86	103.87
18	66.63	58.12	62.37	47	8.50	4.25	12.75	10.63	4.24	.00	10.63	6.38	31.88	4.24	.00	70.21	74.45	119.08
11	58.50	61.30	59.90	44	6.81	9.09	15.90	4.54	4.54	4.54	4.54	.00	18.16	2.27	.00	65.91	68.18	102.24
3	62.05	56.72	59.38	45	4.44	4.44	8.88	6.66	11.11	.00	4.44	.00	22.21	2.22	.00	80.00	82.22	113.31
Av	65.64	60.15	62.90		8.96	4.91	13.87	6.19	5.19	1.08	5.20	1.70	19.36	4.03	.00	72.01	76.04	109.28

TABLE 2.—*Coefficients of correlation between yields and percentages of progenies segregating for different defects in 19 varieties of corn*

Kind of defects	Coefficients of correlation with	
	1925 yield	Two-year average yield
Seed:		
Endosperm	−0.0431±0.1545	−0.1454±0.1515
Germ	−.2627 ± .1437	−.2218 ± .1471
Total	−.1511 ± .1512	−.1609 ± .1507
Seedling:		
White	.0405 ± .1545	.0275 ± .1546
Striped	−.1595 ± .1509	−.2964 ± .1412
Virescent	.1186 ± .1525	.1121 ± .1528
Yellow	−.2931 ± .1415	−.2473 ± .1453
Pale green	.1908 ± .1491	.1962 ± .1488
Total	−.0441 ± .1544	−.0864 ± .1536
Plant:		
Dwarf	.1623 ± .1507	.2992 ± .1409
Golden	.5063 ± .1151	.5711 ± .1043
Barren	−.3319 ± .1377	−.3401 ± .1369
Total	−.2646 ± .1439	−.2441 ± .1455
All defects	−.2365 ± .1460	−.2491 ± .1451

Coefficients of correlation calculated between the percentages of progenies segregating for defects and the yields of the 19 varieties are recorded in Table 2. These correlations substantiate the conclusions drawn from the data in Table 1. The same five abnormalities show a negative correlation with yield and the remaining five a positive correlation. The correlation between golden and yield appears of statistical significance, but Table 1 shows that only three of the varieties segregated for golden. With only three entries in the correlation table this coefficient can not be considered significant. With this one exception none of the coefficients are three times their probable errors. The correlation between the number of ears segregating for barren plants and yield is the most significant statistically in that it is nearly three times its probable error. It is of interest to note that the totals under each of the three classes of defects as well as the total for all defects are negatively correlated with yield.

DISCUSSION

It is hard to believe that abnormalities such as those studied in this problem should have as little effect upon the yield of commercial open-pollinated varieties of corn as the data obtained indicate. It would seem that even though these recessive abnormalities appear relatively infrequently in the homozygous condition, they would have some effect upon vigor and yield when present in the heterozygous condition in the numbers observed in these 19 varieties. This apparent lack of deleterious effect might be easy to understand by assuming complete dominance of the normal allelomorph of each of the defects studied. Such an assumption, however, would not be justified in the light of our present knowledge of genetics.

Lindstrom (*4*) has pointed out that the defects appearing in such large numbers in corn are distributed over the chromosomes in such

a way that it might be difficult to discard factors causing the defects without at the same time discarding important yield factors. Mangelsdorf (*6*) in making a study of the effect of an endosperm defect upon yield, eliminated the factor of linkage with yield factors by comparing yields of plants known to be similar in all genetic factors excepting the one causing the defect. He obtained only slight differences in favor of the homozygous normal plants but the differences in vigor as judged by different criteria were rather consistent. It may be that when more data are obtained from tests such as that of Mangelsdorf, it will be found that some of the defects, when considered independently of closely linked factors, do have a decided effect upon vigor of growth and yield.

One might wonder at the large numbers of the recessive defects present in commercial varieties of corn. It is true that these recessive lethals are carried in the strain in the heterozygous condition due to broad cross-pollination and extreme heterozygosity in corn. However, some explanation seems necessary to account for the large numbers of lethals other than the mere fact that they can be carried for some generations in the heterozygous condition.

Lindstrom (*4*) has suggested that it may be difficult to discard the genetic factors causing these deleterious characters from our commercial varieties on account of linkages with important factors for yield. Might this not also explain the large numbers of defects? It is easy to imagine the factor causing one of these defects to be linked with an important yield or vigor factor and thereby be carried almost indefinitely in the strain. If this is the case, defects caused by factors linked with important yield factors would be carried along in the strain and those linked with less important yield factors would tend to be eliminated. If such a phenomenon is assumed, it is easy to see that some of our best varieties might contain large numbers of the lethal defects even though the defects might be deleterious in the heterozygous condition.

Mangelsdorf (*5*) has offered an explanation for the accumulation of lethal factors by assuming them to be linked with other deleterious factors, thus tending to keep short sections of the chromosomes which bear them in an enforced state of heterozygosity. He refers to the work of Muller (*7*) in which it is demonstrated that balanced lethal factors afford protection to other mutative changes. This of course is a theoretical situation which occurs occasionally, but one could scarcely expect that any appreciable number of the hundreds of defects would thus be balanced.

SUMMARY

Experimental data obtained from plantings of selfed lines of 19 varieties of corn indicate that there is no statistically significant relation between numbers of defects present and yield. When the defects were divided into the three classes—seed, seedling, and plant—it was observed that the totals under each of the three classes, as well as the total for all defects, were negatively correlated with yield. This might indicate a slight deleterious effect of the groups of defects upon yield, but the correlation coefficients were none of them large enough to be considered significant. In the light of our present knowledge of genetics it is difficult to explain why there is such a lack of deleterious effects of abnormalities upon yield in corn.

LITERATURE CITED

(1) COLLINS, G. N.
1926. A COMPARISON OF MAIZE-BREEDING METHODS. U. S. Dept. Agr. Bul. 1396, 22 p., illus.

(2) GARBER, R. J., and ROWLEY, H. K.
1927. A DEFECTIVE ENDOSPERM IN THE HETEROZYGOUS CONDITION AS RELATED TO YIELD IN MAIZE. Jour. Amer. Soc. Agron. 19: 797–803.

(3) KYLE, C. H., and STONEBERG, H. F.
1925. ASSOCIATIONS BETWEEN NUMBER OF KERNEL ROWS, PRODUCTIVENESS, AND DELETERIOUS CHARACTERS IN CORN. Jour. Agr. Research 31: 83–99.

(4) LINDSTROM, E. W.
1920. CHOLOROPHYLL FACTORS OF MAIZE. Jour. Heredity 11: [269]–277, illus.

(5) MANGELSDORF, P. C.
1926. THE GENETICS AND MORPHOLOGY OF SOME ENDOSPERM CHARACTERS IN MAIZE. Conn. Agr. Expt. Sta. Bul. 279, p. 513–614, illus.

(6) ———
1928. THE EFFECTS OF A LETHAL ON THE HETEROZYGOTE IN MAIZE. Jour. Heredity 19: [123]–131, illus.

(7) MULLER, H. J.
1918. GENETIC VARIABILITY, TWIN HYBRIDS AND CONSTANT HYBRIDS, IN A CASE OF BALANCED LETHAL FACTORS. Genetics 3: [422]–499, illus.

(8) WENTZ, J. B., and STEWART, R. T.
1927. EFFECT OF A SEMI-LETHAL FACTOR UPON YIELD IN SOYBEANS WHEN PRESENT IN THE HETEROZYGOUS CONDITION. Jour. Amer. Soc. Agron 19: 850–853.

THE INHERITANCE OF EGG WEIGHT IN THE DOMESTIC FOWL[1]

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INTRODUCTION

Egg weight is of vital concern to the poultry breeder because of its economic importance. In the first place, large eggs produce large chicks, as has been shown by Benjamin (*3*),[2] Halbersleben and Mussehl (*8*), Jull (*9*), Upp (*12*), and others. In the second place, large eggs command a higher price and small eggs are rigidly discriminated against in market grading. These factors, together with the possibility of superior vigor in chicks hatched from large eggs (*3*), have created a strong demand for large eggs in poultry-breeding operations.

Commercially, hens' eggs are classified as "standard" in weight if they average 24 ounces per dozen (56.7 gm. each), with no individual eggs weighing less than 22 ounces per dozen. Ordinary laying hens will attain this standard. Early sexual maturity, so necessary for maximum annual egg production, is intimately correlated with small egg size, as shown by the work of Dryden (*5*), Hadley and Caldwell (*7*), Lippincott (*11*), Atwood (*1*), and Jull (*10*). Under existing conditions it becomes necessary to establish by genetic methods a race of fowls that will combine early sexual maturity and large egg size.

RELATION BETWEEN AGE OF BIRDS AND WEIGHT OF EGGS

In studies on the inheritance of egg weight it is necessary to discover the relation between age of birds and weight of eggs and also to ascertain at what age maximum egg size is attained.

Published data showing the progressive increase in egg weights by months during the pullet laying year are very meager for heavy-producing strains. Curtis's (*4*) work with Barred Plymouth Rocks shows a progressive increase in egg weight from the time the first pullet egg was laid to the month of April. Her birds did not, however, reach the "standard" egg weight until the beginning of the second laying year.

Hadley and Caldwell (*7*) present data regarding egg weight in a flock of White Plymouth Rocks not bred for heavy egg laying. Their records indicate that maximum egg weight is attained during the second laying year. Atwood (*2*), in his experiments with White Leghorns, found that maximum egg weight is not reached until the third laying year.

Records showing the weight of the eggs produced by 105 Rhode Island Red females hatched in 1927 have been kept at this station.

[1] Received for publication Dec. 17, 1928; issued May, 1929. Contribution No. 82 from the Massachusetts Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 519.

Each egg was weighed as it was taken from the trap nest and the records were kept from the time the first pullet egg was laid up to January 1. The eggs from each individual were again weighed from February 22 to March 3, inclusive. The data show that a pullet must lay eggs averaging 52 gm. in weight for the month of November in order to be laying eggs of "standard" weight (56.7 gm.) by March 1. A pullet's eggs must average 55 gm. in weight for December if she is to lay "standard" eggs by March 1. The eggs of a pullet must average 52 gm. for the period from first egg to January 1 if she is to attain "standard" egg weight by March 1.

Data taken at this station on Rhode Island Reds bred for heavy egg production are given in Table 1. Egg weights of this flock cover only a 10-day period each year from February 22 to March 3. These weights were therefore taken about one month too early to record the maximum egg weight for the pullet laying year, according to Curtis (*4*), Hadley (*6*), and Atwood (*2*). Only the same individuals are compared from year to year.

TABLE 1.—*Mean weight in grams of eggs laid by the same hens during the 10-day periods, February 22 to March 3, of successive year*

Series	Number of hens	Mean weight of eggs during the 10-day period in—			
		Pullet year	Second year	Third year	Fourth year
E	17	56.73	60.32		
	9	56.58	59.53	58.31	
	3	55.67	56.03	55.78	56.31
F	23	56.15	60.08		
	5	54.33	60.48	60.33	
G	27	55.26	60.62		
Totals	67	55.94	60.36		
	14	55.78	59.87	59.03	
	3	55.67	56.03	55.78	56.31

From these data, it appears probable that mature egg weight is attained in production-bred Rhode Island Reds during the second laying year and that egg weight does not increase after the second year.

INHERITANCE STUDIES

The eggs from a flock of 526 Rhode Island Red pullets hatched in 1927 were weighed as they were taken from the trap nest. The weights, which were recorded to the nearest gram, were kept from the time the first pullet egg was laid until January 1. All birds used had been bred for 14 years exclusively for characteristics concerned in heavy egg production. They may therefore safely be classed as production bred.

In studying the mode of inheritance of egg weight, five sires were chosen that had the greatest number of daughters hatched in 1927 for which egg-weight records were available. These males were mated to 18 dams. In the resulting progenies the number of daughters ranged from 7 to 18, with a total of 224. Smaller families were omitted because of unreliability in ratios of egg weight.

In the studies reported here egg weight in grams is taken as the measure of egg size. For this reason, the terms "egg weight" and "egg size" will be used interchangeably. The data collected in these studies are shown in the form of graphs. (Figs. 1–6.)

Figure 1 is a frequency-distribution curve showing the mean egg weight for 526 birds from the date the first pullet egg was laid to January 1. In a general way, the graph conforms to a normal frequency-distribution curve, but indications of a multimodal condition are apparent. This fact suggests that egg weight must depend upon several heritable factors. The mean for the entire population is 49.7 gm. (21 ounces to the dozen). The modal class is also in very

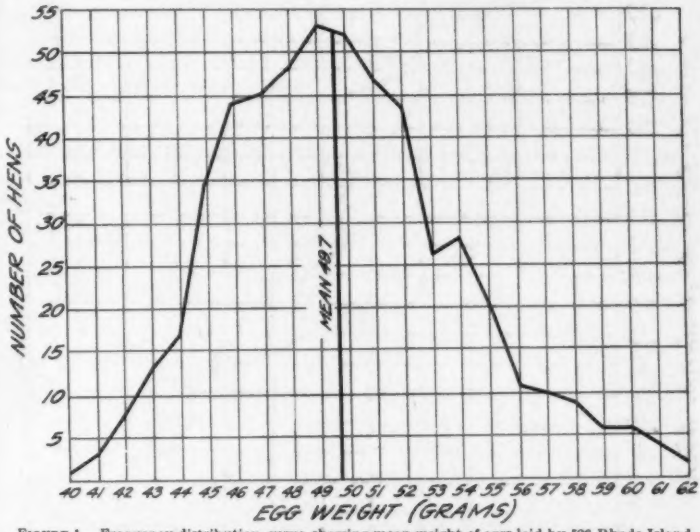

FIGURE 1.—Frequency-distribution curve showing mean weight of eggs laid by 526 Rhode Island Red hens from the date each pullet laid her first egg to January 1

close proximity to the mean. The graph clearly indicates that the majority of the flock lack the necessary 52-gm. weight to bring the egg weight to a standard of 56.7 gm. by March 1. More than half of this group of pullets falls below the 52-gm. weight that would place them in the "standard" class of spring layers.

Figure 2 illustrates graphically the relation between number of eggs laid before January 1 and the mean weight of eggs laid during the same period. This chart clearly shows that the greater the number of eggs laid up to January 1, the smaller the mean size of the eggs. The graph in Figure 2 rather closely approaches a straight line. The coefficient of correlation between number of eggs laid to January 1 and the mean weight of eggs to January 1 is -0.3673 ± 0.0254. In the flock studied there is therefore a significant moderate degree of correlation between number of eggs laid before January 1 and their mean weight. Using the regression coefficient, it is found that those birds averaging 52-gm. eggs might be expected to lay 43.47 eggs during the period, while the actual figure was 42.93 eggs. These data,

therefore, appear to indicate that heavy winter egg laying shows a tendency to be accompanied by small egg size.

Figure 3, which presents a rather common frequency distribution, shows the mean egg weight of the 13 daughters of female G1433 to January 1. This graph shows four common phenotypes for egg size; namely, (1) 47 gm. or under, (2) 48 to 49 gm., (3) 50 to 51 gm., and (4) 52 gm. or over.

Figure 4 records the frequency distribution of the 11 daughters of female G991 with respect to mean egg weight. This graph shows two small egg phenotypes and the large egg phenotype.

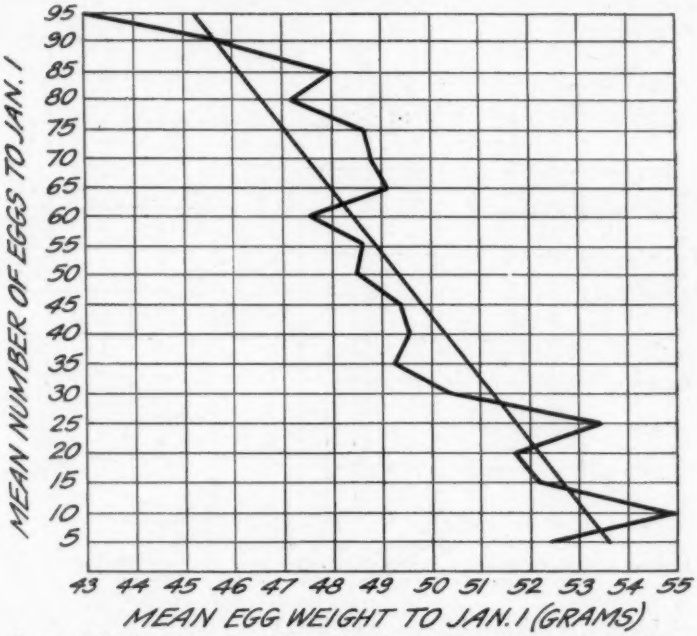

FIGURE 2.—Relation between the number and size of eggs laid by 526 Rhode Island Red hens from the date each pullet laid her first egg to January 1

Figure 5 shows a similar frequency distribution for the 13 daughters of female G997 in egg weight to January 1. Here again two small egg phenotypes appear beside the large egg group.

Figure 6 shows the distribution of the 17 daughters of female G1383 with respect to egg weight. This particular dam laid a very small egg (46.7 gm.) during the hatching season. Her daughters fall into three groups in the small-egg class. She had one daughter that laid 57-gm. eggs and another that laid 51-gm. eggs. It seems probable that both of these daughters really represent "standard" egg types. The genetic formula of this hen is probably *Aabb*. Her 17 daughters gave the following ratio for egg size: 10:5:1:1; expected ratio, 8.5:6.4:0:2.1.

Many more illustrations of the distribution of full sisters with respect to mean-egg weight to January 1 might be presented. All distributions from 18 different dams show a definite grouping into three small-egg classes and a distinct large-egg class. It is believed, therefore, that the graphs present a clear picture of the situation from the standpoint of heredity.

DISCUSSION

The wild ancestor, *Gallus bankiva*, of the domestic hen lays a very small egg, probably not exceeding 40 gm. On the other hand, most of the present-day pure breeds of poultry produce eggs rather closely approximating 56.7 gm., but the number of eggs is not great

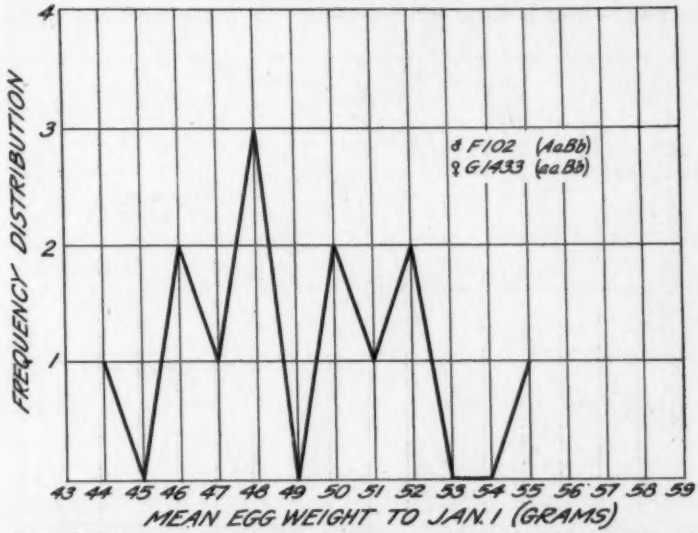
FREQUENCY DISTRIBUTION
4
3
2
1
♂ F102 (AaBb)
♀ G1433 (aaBb)
43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59
MEAN EGG WEIGHT TO JAN. 1 (GRAMS)

FIGURE 3.—Frequency-distribution curve showing mean weight of eggs laid by 13 daughters of G1433 from the date each pullet laid her first egg to January 1

unless the hens are bred for heavy laying. Very frequently small-egg size is a concomitant of heavy laying, particularly in early maturing flocks, as Jull (*10*) has pointed out. What is the situation from the genetic standpoint?

It appears probable that in the evolution of modern breeds of poultry, a gene for large-egg size (*B*) has been reasonably well "fixed" by breeders, so that the average hen now lays a moderate number of large eggs but is not necessarily homozygous for gene *B*. These data also indicate that minor modifiers of egg size are operating, so that the range above 52 gm. as shown by these data is rather wide. These modifiers may be both physiological and hereditary, but are of minor significance in comparison with gene *B*. In the second place, a dominant gene (*A*) for small-egg size (below 56.7 gm. by March 1) has appeared. The gene *A* behaves as epistatic to gene *B*, consequently those individuals carrying both genes *A* and *B* will lay small eggs. Those birds carrying *A* alone lay smaller eggs than those

carrying both genes (*A*, *B* birds), and those lacking both (*aabb* birds) lay very small eggs. Only *aB* birds lay "standard" eggs.

The probable genotypes for egg size are as follows:

aabb, *AAbb*, *Aabb* } 47 gm. or under up to January 1.
AABb, *AaBb* } 48 to 49 gm. up to January 1.
AABB, *AaBB* } 50 to 51 gm. up to January 1.
aaBB, *aaBb* } 52 gm. or over up to January 1.

These represent the four major classes of pullets with respect to egg weight used in these studies. Class 1 is made up of those pullets

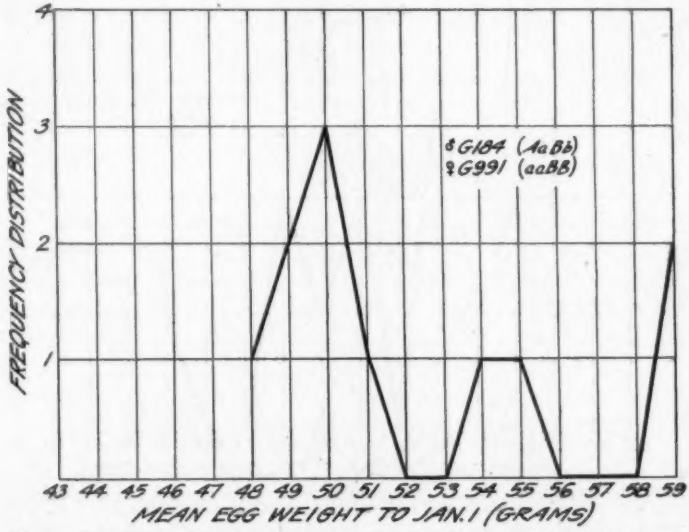

FIGURE 4.—Frequency-distribution curve showing mean weight of eggs laid by 11 daughters of G991 from the date each pullet laid her first egg to January 1

that lay eggs weighing 47 gm. or under up to January 1. The range in this group is wide because it is made up of three genotypes. In class 2 the range is 48 to 49 gm. Class 3 has a range from 50 to 51 gm. Class 4 includes all pullets whose eggs averaged 52 gm. or more up to January 1. This last class ranges from 52 to 60 gm., and this wide range is evidently produced by several variables, only one of which (dominant gene *B*) has been considered in these studies. For the purpose of this work, namely, to discover the major genetic differences between birds laying 56.7 gm. eggs by March 1 and those laying eggs smaller than 56.7 gm., it seems justifiable to refer to the above four classes of layers as phenotypes (recognizable classes). In this report, therefore, but one large-egg class is considered, while three small-egg classes are discussed. The fact that gene *A* is epistatic to gene *B* makes the consideration of the three small-egg classes of vital

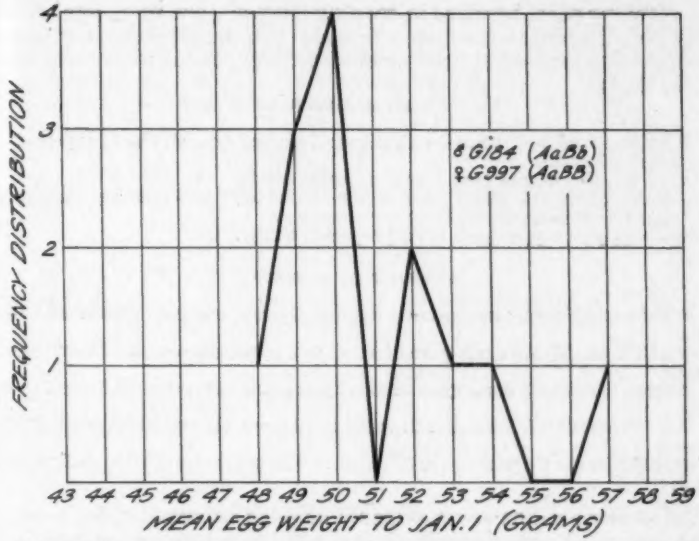

FIGURE 5.—Frequency-distribution curve showing mean weight of eggs laid by 13 daughters of G997 from the date each pullet laid her first egg to January 1

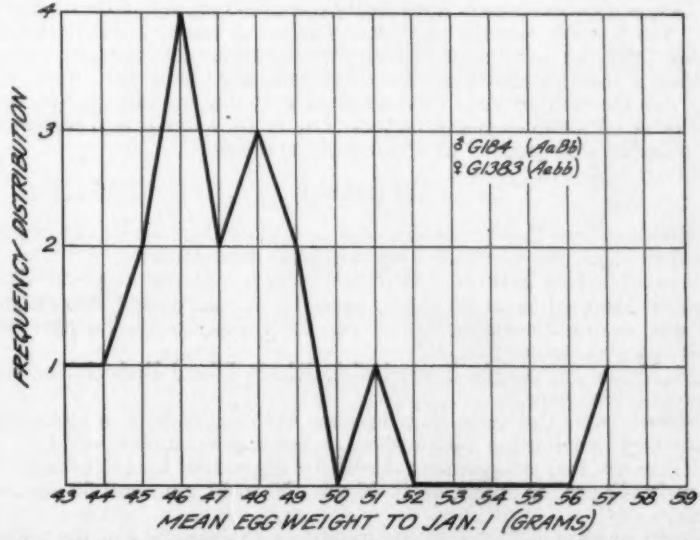
FREQUENCY DISTRIBUTION
4
3
2
1
♂ G184 (AaBb)
♀ G1383 (Aabb)
43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59
MEAN EGG WEIGHT TO JAN. 1 (GRAMS)

FIGURE 6.—Frequency-distribution curve showing mean weight of eggs laid by 17 daughters of G1383 from the date each pullet laid her first egg to January 1

concern in breeding operations. It is this group of birds that indicates the blending effect reported in crossing large-egg and small-egg breeds. This blending or intermediate effect also is to be expected in progeny from mating large-egg hens to males carrying gene *A* within the same flock, thus:

TYPICAL MATINGS

♂ F102 (*AaBb*) × ♀ E319 (*AABb*) gave 13 daughters, all small; expected 13 daughters, all small.

Small egg phenotypes gave 7:4:2; expected 5:6:2.

♂ G980 (*aaBb*) × ♀ F2238 (*AaBB*) gave 5 small-egg to 6 large-egg daughters; expected 5½ small-egg to 5½ large-egg daughters.

Small-egg phenotypes gave 0:2:3; expected 0:2½:2½.

SUMMARY OF MATINGS

♂ F102 (*AaBb*) × 4 dams gave 34 small to 7 large; expected 32.6 small to 8.4 large.

♂ F1372 (*AaBb*) × 3 dams gave 31 small to 3 large; expected 29.7 small to 4.3 large.

♂ G148 (*AaBb*) × 3 dams gave 32 small to 11 large; expected 36.5 small to 6.5 large.

♂ G980 (*aaBb*) × 5 dams gave 21 small to 42 large; expected 17.8 small to 45.2 large.

♂ G1440 (*aaBb*) × 3 dams gave 34 small to 9 large; expected 32.8 small to 10.2 large.

All of these ratios are reasonably close to expectation for a small population. To develop a flock that will breed true for large-egg size it therefore becomes necessary to make the flock homozygous for gene *B*, using only female breeders laying 56.7 gm. or larger eggs and mating these to males that breed true for the large-egg gene.

Large-egg size alone is apparently a simple characteristic to breed for, yet possible linkage relations of genes *A* and *B* with desirable genes from the standpoint of high fecundity may be such as to complicate a breeding program where maximum egg production and large-egg size are both desired. Future work may disclose linkage between factor *A* for small eggs and factors *E* or *E'* for early sexual maturity or possibly with genes *I* or *I'* for high intensity.

SUMMARY

Rhode Island Red pullets showed a marked increase in size of egg from the time the first pullet egg was laid up to March 1.

Available data indicate that pullets to attain an egg weight of 56.7 gm. by March 1 must lay eggs averaging 52 gm. during November, 55 gm. during December, or 52 gm. for the entire period between first egg and January 1.

Maximum egg weight is reached in Rhode Island Reds during the second laying year.

Sisters from the same dam arrange themselves in a multimodal frequency distribution with respect to egg size up to January 1.

There are four conspicuous classes for egg weight up to January 1; namely, 47 gm. or under, 48 to 49 gm., 50 to 51 gm., and 52 gm. or over.

Birds averaging 52 gm. in egg weight up to January 1 of the pullet year may be classed as large egg pullets because they will produce eggs weighing 56.7 gm. by March 1.

Standard eggs weighing 56.7 gm. or 24 ounces per dozen appear to be made possible by a dominant gene *B* which is not sex linked and is probably cumulative in effect.

Small eggs in improved flocks appear to be caused by a dominant autosexual gene *A*. Gene *A* is epistatic to gene *B* and all birds carrying both genes as well as those carrying *A* alone lay small eggs.

The smallest eggs are laid by pure recessive individuals (*ab* birds), the next in size by *Ab* birds, eggs just under "standard" by *AB* birds, and standard weight eggs by *aB* birds.

Breeding for egg size alone without regard to number of eggs appears to be a simple procedure.

LITERATURE CITED

(1) ATWOOD, H.
1923. CERTAIN CORRELATIONS IN THE WEIGHT AND NUMBER OF EGGS AND THE WEIGHT OF FOWLS. W. Va. Expt. Sta. Bul. 182, 16 p., illus.

(2) ———
1926. SOME FACTORS AFFECTING THE WEIGHT OF EGGS. W. Va. Agr. Expt. Sta. Bul. 201, 30 p.

(3) BENJAMIN, E. W.
1920. A STUDY OF SELECTIONS FOR THE SIZE, SHAPE, AND COLOR OF HENS' EGGS. N. Y. Cornell Agr. Expt. Sta. Mem. 31, p. 191–312, illus.

(4) CURTIS, M. R.
1914. FACTORS INFLUENCING THE SIZE, SHAPE, AND PHYSICAL CONSTITUTION OF THE EGG OF THE DOMESTIC FOWL. Maine Agr. Expt. Sta. Bul. 228, 136 p.

(5) DRYDEN, J.
1899. POULTRY EXPERIMENTS. Utah Agr. Expt. Sta. Bul. 60, p. [133]–151, illus.

(6) HADLEY, P
1919. EGG-WEIGHT AS A CRITERION OF NUMERICAL PRODUCTION IN THE DOMESTIC FOWL. Amer. Nat. 53: 377–393, illus.

(7) ——— and CALDWELL, D. W.
1920. STUDIES ON THE INHERITANCE OF EGG-WEIGHT. I. NORMAL DISTRIBUTION OF EGG-WEIGHT. R. I. Agr. Expt. Sta. Bul. 181, 64 p., illus.

(8) HALBERSLEBEN, D. L., and MUSSEHL, F. E.
1922. RELATION OF EGG WEIGHT TO CHICK WEIGHT AT HATCHING. Poultry Sci. 1: [143]–144.

(9) JULL, M. A.
1923. DIFFERENTIAL SEX GROWTH CURVES IN BARRED PLYMOUTH ROCK CHICKS. Sci. Agr. 4: 58–65, illus.

(10) ———
1924. EGG WEIGHT IN RELATION TO PRODUCTION. PART II.—THE NATURE AND CAUSES OF CHANGES IN EGG WEIGHT IN RELATION TO ANNUAL PRODUCTION IN PULLETS. Poultry Sci. 3: 153–172, illus.

(11) LIPPINCOTT, W. A.
1921. PRELIMINARY NOTE ON THE CORRELATION BETWEEN AGE AT FIRST LAYING AND SIZE OF FIRST EGG IN PULLETS. Jour. Amer. Assoc. Instr. and Invest. Poultry Husb. 7: 73–74.

(12) UPP, C. W.
1928. EGG WEIGHT, DAY-OLD CHICK WEIGHT AND RATE OF GROWTH IN SINGLE COMB RHODE ISLAND RED CHICKS. Poultry Sci. 7: 151–155.

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